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CONTENTS

ADRENALIN AND THE TONUS OF DECEREBRATE RIGIDITY. *Eugene L. Porter* 495

THE CAUSE OF THE DELAYED CLOTTING OF HEMOPHILIC BLOOD. *W. H. Howell and E. B. Cekada* 500

THE PREPARATION AND PROPERTIES OF PROTHROMBIN. *Emil B. Cekada* 512

THE FEMALE SEX CYCLE AS A FACTOR IN LEARNING IN THE RAT. *Josephine Ball* 533

EXPERIMENTAL ANALYSIS OF THE SIMPLE ACTION POTENTIAL WAVE IN NERVE BY THE CATHODE RAY OSCILLOGRAPH. *Joseph Erlanger, G. H. Bishop and H. S. Gasser* 537

THE ACTION POTENTIAL WAVES TRANSMITTED BETWEEN THE SCIATIC NERVE AND ITS SPINAL ROOTS. *Joseph Erlanger, G. H. Bishop and H. S. Gasser* 574

DISTORTION OF ACTION POTENTIALS AS RECORDED FROM THE NERVE SURFACE. *Geo. H. Bishop, Joseph Erlanger and H. S. Gasser* 592

THE REGULATION OF RESPIRATION. IV. TISSUE ACIDITY, BLOOD ACIDITY AND PULMONARY VENTILATION. A STUDY OF THE EFFECTS OF SEMIPERMEABILITY OF MEMBRANES AND THE BUFFERING ACTION OF TISSUES WITH THE CONTINUOUS METHOD OF RECORDING CHANGES IN ACIDITY. *Robert Geselli and Alrick B. Hertzman* 610

THE EFFECTS OF POLARIZATION UPON THE ACTIVITY OF VERTEBRATE NERVE. *G. H. Bishop and Joseph Erlanger* 630

THE ABSORPTION OF BILE PIGMENT FROM THE INTESTINE. *Jesse L. Bollman, Charles Sheard and Frank C. Mann* 658

STUDIES IN AVIAN DIABETES AND GLYCOSURIA. *Theodore Koppányi, Andrew C. Ivy, Arthur L. Tatum and Frederick T. Jung* 666

III. MUSCLE HEMOGLOBIN AS A SOURCE OF BILE PIGMENT. *G. H. Whipple and F. S. Robscheit-Robbins* 675

STUDIES ON ADRENAL INSUFFICIENCY IN DOGS. I. CONTROL ANIMALS NOT SUBJECTED TO ANY TREATMENT. *J. M. Rogoff and G. N. Stewart* 683

STUDIES ON ADRENAL INSUFFICIENCY IN DOGS. II. BLOOD STUDIES IN CONTROL ANIMALS NOT SUBJECTED TO TREATMENT. *J. M. Rogoff and G. N. Stewart* 711

INDEX 731

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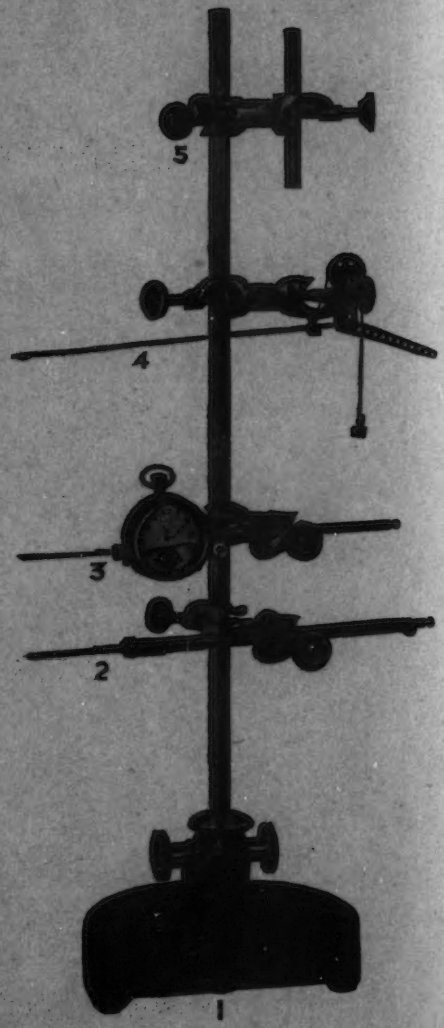

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ADRENALIN AND THE TONUS OF DECEREBRATE RIGIDITY

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If the sympathetic system is responsible for a part or the whole of tonic contraction[1] such as occurs in decerebrate rigidity, it ought to be possible to produce such tonic contraction, or increase it, if present, by the use of adrenalin. The only structures innervated by the sympathetic system which fail to be influenced by adrenalin are the sweat glands, and this apparent exception may not in the end prove to be a real one (Langley, 1921). Various investigators have accordingly attempted to obtain an effect on tonus by injections of adrenalin. I will refer to but two, illustrative of two types of procedure. Gayda (1924) tried to alter an existing tonus. Using the decerebrate cat he noted the position assumed by the extended hind legs and injected adrenalin into one leg in the expectation of observing a difference in position between this leg and the other, indicating increased tonic contraction, but found none. Kuno (1914) attempted to produce tonic contraction in the quiescent frog by injections of adrenalin but was unable to do so.

In contrast to these results, we have the undoubted fact that adrenalin improves the contraction of striated muscle when the muscle is being stimulated artificially (Gruber, 1922). This improvement is particularly striking if the muscle has been fatigued. Gruber found as much as 150 per cent improvement under these circumstances. Gruber is convinced that he has ruled out circulatory alteration and believes that adrenalin improves the contraction by rendering muscle fibers more irritable than before the drug is administered. In view of the all-or-none behavior of both nerve and muscle, this must be interpreted to mean that the muscle fiber which has been rendered incapable of being stimulated by a nerve impulse is enabled by adrenalin to receive such stimulus and respond to it.

[1] I shall not attempt to review the literature on the sympathetic innervation of striated muscle. The reader is referred to such recent papers as Cobb (1925) or Ranson (1926) for summaries and bibliographies.

Its contraction added to the contraction of fibers still functioning, gives the observed improvement in the contraction height.

It would seem logical then to suppose that muscle in tonic contraction might similarly show an improvement following adrenalin, and especially if the injection were made when the tonus curve had been falling for some time; in other words, when the tonus was fatigued. In Gruber's experiments the fatigue is entirely peripheral. In the fatigue of a tonically contracting muscle it is presumably both central and peripheral. But it would seem reasonable to suppose that enough of the fatigue is peripheral to render it possible to improve contraction by adrenalin. It should be borne in mind, however, that Gruber obtains improvement whether the muscle be fatigued or not. The only essentials are that the muscle be responding to stimuli at the time and be capable of better contraction than it is then giving. The tonically contracting muscle which is slowly diminishing in contraction height fulfils these conditions, except that it is being stimulated naturally instead of artificially.

Attempts to cause contraction of a resting muscle by adrenalin may fail (Kuno, 1914) because adrenalin may be unable to initiate stimulation, but only sensitize the muscle to stimuli already coming to it. On the other hand, a muscle in steady and vigorous tonic contraction may be incapable of further improvement (Gayda, 1924) because already contracting to its maximum. In the muscle, however, which is showing a gradually diminishing tonus we might logically look for conditions to be at the optimum for the demonstration of an adrenalin effect. It is justifiable to regard such a muscle as likely to be a more delicate indicator than either the resting or the strongly contracting one. In the graphic record a positive result would be indicated by the change in the shape of the fatigue curve, either an upward movement of the pointer, or at least a distinct lessening in its rate of descent toward the base line.

The present research is based on the assumptions of the foregoing paragraphs.

Method and results. In studying minute changes in the character of muscle contraction, the smaller the muscle the better. Alterations in contraction height are then likely to represent larger percentages of total possible contraction. It was for this reason in a previous research (Porter and Hart, 1923) that we utilized the tenuissimus muscle of the cat. Tenuissimus, however, is a flexor muscle and does not take part in the tonus of decerebrate rigidity. None of the other leg muscles seemed sufficiently small to meet the needs of the present investigation. The next best appeared to be the small ones elevating the tail, although elevation of the tail is by no means so constant a phenomenon in decerebrate animals as extension of the legs. The investigation has been carried out on one of these muscles. It has usually been found possible to produce extension of the

tail when it is not already present. Only very occasionally has it been necessary to discontinue an experiment because of complete failure of the tail muscle to respond.

Cats have been decerebrated by Sherrington's guillotine method (Sherrington, 1915). The muscle employed is one of a large number grouped under the name of extensor caudae lateralis. That one is selected which has its origin opposite the crest of the ilium. Immobilization is secured by the use of a specially designed clamp which has roughly the shape of a two-pronged fork, sharply pointed. It is separated from end to end into two halves which can be screwed together, each half bearing a prong. In applying it, one prong is thrust through the muscle just caudad of the hip joint and is brought to lie along-side the centra of the vertebrae. The other prong is thrust through the fascia of the muscles of the dorsal side of the vertebral column and is made to take a position parallel to and in contact with the spinous processes. The two halves of the clamp are now screwed tightly together, holding the vertebral column rigidly. The rigidity in the leg on the side on which the clamp is applied is diminished or lost, but there is no detectable effect on the other leg or the tail, which can be made to extend as if the clamp were not present. In exceptional cases respiratory movements appear as minute waves in the line written by the muscle on the drum but in the majority of cases the line is a practically straight one when the muscle is at rest.

The cat is supported in a cradle or form of wood with the legs and tail hanging freely in their natural positions. The head is supported horizontally. The clamp on the vertebral column is fastened firmly to the wooden support. The tendon of the muscle is connected by a thread with a light muscle lever magnifying about thirteen times. Blood pressure has been recorded from the carotid artery. A rapid injection of 1 cc. 1:10,000 adrenalin (Parke, Davis) in saline has been the usual procedure. With this strength (Gruber, 1922) obtained marked improvement in contraction of either fatigued or non-fatigued muscles. As a matter of fact his results were positive with doses running all the way from 1 cc. of a 1:100,000 solution to 5 cc. of a 1:1000 solution.

A 1:10,000 solution is well within the limits of dilution at which vaso-dilatation is said to occur within the muscle. Gunning (1917) finds that the strength must be ten to twenty times that in order to produce vaso-constriction instead of vaso-dilatation in the muscle vessels of the dog.

Injections were made into the external jugular vein. The tail can usually be made to extend at will by rubbing the fur on the tail the wrong way. Often extension is vigorous and prolonged from slight stimulation. The amount of contraction shown by the muscle and its duration vary widely. Usually the muscle can be held in steady contraction for many minutes by continuing the irritation of the tail. Occasionally also the tail

is extended spontaneously. In all cases upon discontinuing the stimulation the tail slowly sinks and the muscle record on the drum shows a slow but steady drop. The first part of relaxation is usually quite rapid, followed by a slow fall of the writing lever occupying many minutes.

At a point where the descent is still fairly rapid an injection of adrenalin is made. Figure 1 shows a record of such an experiment. At *1* the muscle contracted spontaneously. Between *1* and *2* the contraction was maintained for 3 minutes by rubbing the fur of the tail whenever the muscle

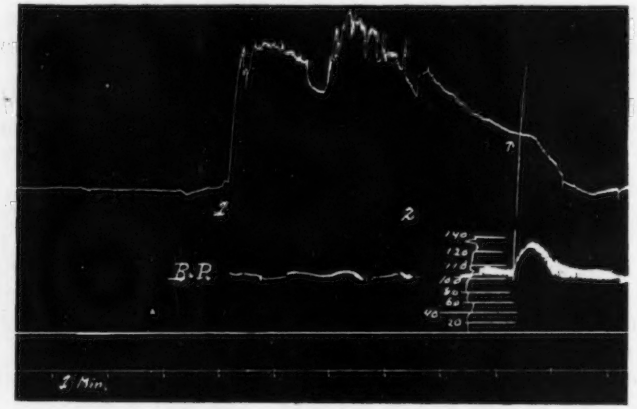

Fig. 1. The effect of an intravenous injection of adrenalin on a fatiguing tonic contraction of one of the extensor muscles of the tail (extensor caudae lateralis) in the decerebrate cat. At *1* spontaneous contraction of the muscle occurred. Between *1* and *2* this contraction was maintained whenever it tended to subside by rubbing the fur of the tail the wrong way. At *2* this stimulation was discontinued and the muscle allowed to relax. At the arrow a rapid injection of 1 cc. of 1:10,000 adrenalin was made into the jugular vein. Note absence of any indication of stimulation by the drug. Lower line: time in minutes. Next line: zero of blood pressure. Third line: blood pressure by membrane manometer. Ordinate crossing upper record indicates moment blood pressure began to rise. The minute undulations are due to respiratory movements.

showed signs of relaxation. At *2* such stimulation was discontinued and 2 minutes later an injection of adrenalin was made as indicated by the arrow. No evidence of stimulation by the drug is detectable. Thirty-five such injections have been made in seven animals and this has been the uniform result without an exception.

The drop in the record a half-minute or so after injection, has appeared often but not always. When it does occur one is inclined to infer that it is due to vaso-constriction and asphyxiation of the muscle as Cannon and Gruber (1916) have suggested. We cannot indeed be sure that the adre-

nalin ever gets past capillary walls and in contact with susceptible tissue. The purpose of this investigation is however merely to demonstrate that, under conditions similar to Gruber's, with the exception of the intact nerve supply, the tonically contracting muscle shows no improvement on injection of adrenalin. To this extent the evidence presented is against the view that the tonus of decerebrate rigidity is dependent on the sympathetic system.

SUMMARY

An artificially stimulated muscle, particularly if fatigued, shows marked improvement in contraction after intravenous injections of adrenalin (Gruber, 1922).

In view of this fact it might be expected that a muscle in tonus would show similar improvement following adrenalin. It would appear probable that this would be most marked if the tonus were diminishing, i.e., fatiguing.

A method is described of using a small extensor muscle of the tail in the decerebrate cat for testing the effect of adrenalin when the muscle is showing steadily decreasing tonus.

If this muscle be thrown into tonic contraction, and then its tonus allowed to diminish, there is no improvement in contraction following intravenous injections of adrenalin (in doses of 1 cc. of a 1:10,000 solution).

It is concluded that this constitutes evidence that the tonic contraction of decerebrate rigidity is not brought about through sympathetic innervation.

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THE CAUSE OF THE DELAYED CLOTTING OF HEMOPHILIC BLOOD

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It has been shown that the characteristic feature of hemophilic blood is its delayed coagulation time, the difference between it and normal blood being most evident when what may be called the intrinsic coagulability of the blood is alone considered, that is to say, its coagulability when shed without coming into contact with the tissues. Accepting this fact observers have examined the various known factors of coagulation to ascertain whether one or more of them differ in any respect from the same factors as they occur in normal blood. Differences have been described and have served as a basis for an explanation of the cause of hemophilia, or more precisely, as an explanation of the delayed coagulation. In most cases subsequent work has failed to support these specific theories.

There is general agreement among all recent workers that the fibrinogen of hemophilic blood does not differ in either quantity or quality from that of normal blood. Thrombin added to hemophilic blood or to the fibrinogen prepared from it causes clotting in normal time (1), (2), (3), (4), (5). The fibrin formed in the clotting of hemophilic blood is stated to be normal in amount (6). The calcium also is present in usual concentrations. Nolf (7) states that the calcium content does not differ from that of normal blood. Hess (8) finds that in the typical hemophilic the amount of calcium, as determined by McCrudden's method, is identical with that found in ordinary blood.

In the three cases that we have studied the concentration of calcium,[1] estimated by the method of Kramer and Tisdall, fell within the normal range, averaging 10.3 mgm. per 100 cc. of blood. The addition of calcium to hemophilic blood, although it may shorten somewhat the time of clotting, does not by any means restore the normal period. There is therefore no evidence to show that the delayed clotting is due to a deficiency in calcium.

The differences in fibrin factors that have been suggested as existing in

[1] These determinations were kindly done for us by Miss Georgian Adams.

the hemophilic are the presence of anticoagulant in excess, a deficiency in thrombokinase or thromboplastic material, and either a deficiency in amount of the prothrombin or a change in its properties.

Weil (9) seems to have been the first to suggest that the delayed clotting is due to the presence of an inhibitory substance or anticoagulant. He found that in some cases at least the serum from hemophilic blood causes a delay in the clotting of normal blood, but other workers have not confirmed this result. Several workers (Nolf and Herry (10), Addis (1), Howell (5), Minot and Lee (11) et al.) have attempted by different methods to compare normal and hemophilic bloods with reference to their content in antithrombin. The result has been negative, the amount in hemophilia is not definitely increased. Howell (5) has shown that in normal blood there is another inhibitory substance present, designated as heparin, whose inhibitory influence is exerted upon the prothrombin rather than the thrombin and whose function therefore is to prevent or delay the production of thrombin. On the hypothesis that this substance may be present in excess in hemophilic blood he examined the blood of three hemophilic subjects, but was unable to show a distinct increase in heparin. The general theory advanced by Weil has therefore not been substantiated by subsequent work.

Sahli (6) and also Morawitz and Lossen (12) believed that the content in thrombokinase or thromboplastic material of the hemophilic tissues in general or of the blood cells in particular is less than in the normal individual and that this deficiency is the cause of the delay in clotting. But direct examination of hemophilic tissues, in cases that have come to autopsy, reported by Gressot (13), Lowenburg and Rubenstone (14) and Minot and Lee (11) have failed to confirm this theory. With regard to the blood cells themselves Sahli (6) found that suspensions of washed corpuscles of hemophilic blood have less effect in hastening the coagulation of hemophilic blood than similar suspensions of corpuscles from normal blood. But repetitions of these experiments by Addis and by Wöhlisch gave contrary results. Sahli's theory has therefore failed of corroboration at the hands of other investigators.

In recent years the prevalent view in regard to the difference between normal and hemophilic blood has been that in the latter the prothrombin factor is modified. Addis (1) and also Feissly (2) have reported experiments which seem to show that the prothrombin or proserozyme in hemophilic blood is more stable or has a diminished reactivity, and is therefore activated more slowly than under normal conditions. Klinger (4), on the contrary, has suggested that the prothrombin in hemophilic blood is reduced in amount and Howell has offered a similar hypothesis. In the present paper this question has been especially investigated and it may be said at once that the results obtained indicate that the prothrombin of

hemophilic blood does not differ either in quantity or quality from that of normal blood.

METHODS. Prothrombin was prepared from normal and hemophilic blood by three methods.

1. *The acetone method of Howell.* The blood is oxalated and centrifugalized and the clear plasma is pipetted off. To each 5 cc. of plasma are added 5 cc. of acetone. The solutions are well mixed and the precipitate is filtered off quickly by suction and washed once with ether. The filter is removed and opened, and the precipitate spread out in a thin layer by means of a spatula. The filter with its precipitate is dried in a current of air from an electric fan. The dried filters are kept in a desiccator until needed. A prothrombin solution is made by cutting a paper into small pieces and extracting it for 20 to 30 minutes with 10 cc. water to which two drops of a 0.5 per cent solution of sodium bicarbonate have been added. The filtered solution contains the prothrombin.

2. *The heating method.* In this method the oxalated plasma is heated quickly to 56°C. in a water bath and the resulting precipitate of fibrinogen is centrifugalized off. The supernatant liquid contains the prothrombin.

3. *Gratia's method* (15). The oxalated plasma is inoculated with a fresh culture of Staphylococcus aureus and incubated for 6 to 12 hours or longer at 37°C. With the growth of the organism the fibrinogen separates out as a clot which is centrifugalized off, the supernatant liquid containing the prothrombin. If the incubation period is prolonged the clot disintegrates and the fibrinogen settles out as a precipitate. For some reason not apparent the hemophilic plasma required a longer incubation to cause clotting of the fibrinogen.

The fibrinogen solutions used in some of the experiments were obtained by the ammonium sulphate method as used in this laboratory (16). These solutions did not clot spontaneously nor after the addition of calcium chloride. The calcium chloride used for activating the prothrombin was a 0.5 per cent solution, made from crystallized calcium chloride, the strength being determined by titration with potassium permanganate.

The three hemophilic patients used are brothers, Joseph Y., aged 26, Jesse Y., aged 25, and Fred Y., aged 19. They have been under observation for 10 or 12 years, and were reported upon in a previous paper. They were bled at intervals of about a month, 50 to 60 cc. being withdrawn through an arm vein at each bleeding.[2] As part of the study being made the three patients were subjected to different treatments during the time of the experiments to see if it was possible to influence the time of coagulation of their bloods. The following results were obtained upon the coagulation times during this period. It should be stated that the coagulation time was determined by emptying 2 cc. of the blood from the syringe into a clean glass vial 15 mm. diameter. Under these conditions normal blood clots in 5 to 15 minutes. The end point of clotting in hemophilic blood is not so sharp as in normal blood; usually there is first a

[2] We are greatly indebted to Dr. Geo. D. Rowe for his kindness in obtaining these samples of blood and for giving the injections of calcium chloride.

partial or imperfect clot followed some minutes later by gelatinization of the whole mass.

Joseph Y.	1st bleeding,	Nov. 5, 1925.	Clotting time 3 hr. 15 min.
	2nd bleeding,	Dec. 3, 1925.	Clotting time 3 hr.
	3rd bleeding,	Jan. 6, 1926.	Clotting time 2 hr.
	4th bleeding,	Feb. 18, 1926.	Clotting time 2 hr. 15 min.
	5th bleeding,	Mar. 19, 1926.	Clotting time 3 hr.
	6th bleeding,	Apr. 29, 1926.	Clotting time 2 hr. to 2 hr. 15 min.

From November 5, 1925, until February 18, 1926, the patient took a daily dose of cephalin in a glass of water before breakfast. Whether or not the reduction in clotting time from 3 hours to 2 hours was a consequence of this treatment, or whether there are normal variations of this amount is not evident from this record. But it is clear that the treatment was not sufficient to restore clotting time to anything approaching the normal.

Jesse Y.	1st bleeding,	Nov. 5, 1925.	Clotting time 1 hr. 30 min. to 2 hr.
	2nd bleeding,	Dec. 3, 1925.	Clotting time 1 hr. 45 min. to 2 hr.
	3rd bleeding,	Jan. 6, 1926.	Clotting time 1 hr.
	4th bleeding,	Feb. 18, 1926.	Clotting time 1 hr. 30 min. to 1 hr. 45 min.
	5th bleeding,	Mar. 19, 1926.	Clotting time 2 hr. 15 min.
	6th bleeding,	Apr. 29, 1926.	Clotting time 2 hr.

This patient before the first bleeding on November 5, had been receiving intravascular injections of calcium chloride once or twice a month. The dose given was either 20 cc. of a 7 per cent solution or 40 cc. of a 3 per cent solution. On February 18 this treatment was suspended. The bleeding on March 19 was one month after the last injection of calcium chloride and that on April 29 was two months afterward. The records seem to show that the calcium injections did serve to hasten somewhat the clotting time of the blood.

Fred Y.	1st bleeding,	Dec. 3, 1925.	Clotting time 2 hr. 30 min.
	2nd bleeding,	Jan. 6, 1926.	Clotting time 3 hr. 30 min. to 4 hr.
	3rd bleeding,	Feb. 18, 1926.	Clotting time 2 hr. 30 min.
	4th bleeding,	Mar. 19, 1926.	Clotting time 2 hr. 30 min.
	5th bleeding,	Apr. 29, 1926.	Clotting time 2 hr. 45 min.

From December 3 to January 6 this patient had been given a daily dose of cod liver oil with the apparent result that the clotting time was prolonged. This treatment was therefore suspended and from January 10 to February 18 he was given daily doses of cephalin. From February 18 to April 29 there was no treatment.

THE PROTHROMBIN OF HEMOPHILIC BLOOD. In the first series of experiments an effort was made to determine whether the amount of prothrombin in hemophilic blood varies distinctly from that of normal blood

For this purpose preparations of prothrombin from the two bloods were made in different concentrations, and after activation by calcium chloride were used to cause the coagulation of a certain amount of a fibrinogen solution. It was assumed that if the prothrombin in the hemophilic blood was present in smaller concentration than the normal this fact would be revealed at certain dilutions by the longer time required for the clotting of the fibrinogen.

1. Prothrombin prepared from normal and hemophilic blood by the acetone method.

PROTHROMBIN	CALCIUM CHLORIDE	FIBRINOGEN	COAGULATION TIME	
			Normal	Hemophilic
1 drop	3 drops	8 drops	4 hr.	4 hr.
2 drops	3 drops	8 drops	33 min.	25 min.
3 drops	3 drops	8 drops	18 min.	17 min.
4 drops	3 drops	8 drops	15 min.	16 min.
5 drops	3 drops	8 drops	15 min.	16 min.
6 drops	3 drops	8 drops	15 min.	16 min.

2. Prothrombin prepared from normal and hemophilic blood by the heating method.

PROTHROMBIN	CALCIUM CHLORIDE	FIBRINOGEN	COAGULATION TIME	
			Normal	Hemophilic
1 drop	3 drops	7 drops	37 min.	44 min.
2 drops	3 drops	7 drops	37 min.	37 min.
3 drops	3 drops	7 drops	37 min.	32 min.
4 drops	3 drops	7 drops	37 min.	32 min.
5 drops	3 drops	7 drops	37 min.	32 min.

3. Prothrombin prepared from normal and hemophilic blood by Gratia's method.

PROTHROMBIN	CALCIUM CHLORIDE	FIBRINOGEN	COAGULATION TIME		
			Normal	Hemophilic 1	Hemophilic 2
2 drops	5 drops	8 drops	18 min.	14 min.	22 min.
3 drops	5 drops	8 drops	14 min.	12 min.	18 min.
4 drops	5 drops	8 drops	12 min.	12 min.	18 min.
5 drops	5 drops	8 drops	12 min.	12 min.	18 min.
6 drops	5 drops	8 drops	12 min.	12 min.	15 min.
7 drops	5 drops	8 drops	12 min.	12 min.	15 min.

The results of these experiments indicate that the hemophilic blood contains prothrombin in concentrations similar to those occurring in normal blood. The delayed clotting in hemophilia can not be explained on the hypothesis of a diminished quantity of prothrombin.

SECOND SERIES. In this series the purpose held in view was to determine whether or not the prothrombin of hemophilic blood differs qualitatively from that of normal blood as determined by the relative ease of activation. Calcium chloride was added to prothrombin solutions prepared from the oxalated plasma of the two bloods. These mixtures were allowed to stand for different intervals of time from zero to thirty minutes. At the end of each period the calcium was precipitated by excess of oxalate and the thrombic action of the mixture was tested by adding it in similar quantities to a given amount of fibrinogen or oxalated plasma. When the latter was used for the coagulation test enough plasma was added to precipitate the calcium in the prothrombin-calcium chloride mixture. In the case of the fibrinogen solutions it was necessary to add excess of potassium oxalate to the prothrombin-calcium chloride mixture before adding the fibrinogen. This addition of potassium oxalate always resulted in a great prolongation of the coagulation time the reason for which was not clear, since addition of a similar excess of oxalate to a ready formed thrombin solution does not interfere with the coagulative action of the thrombin on fibrinogen.

The experiments developed the fact that the activating effect of calcium chloride on prothrombin is instantaneous, thrombin is formed at once although there may be some delay before the optimum effect is obtained. But in this respect the prothrombin from hemophilic blood behaved in the same way as that from normal blood. The results of several of these experiments are given in detail.

1. *Prothrombin prepared by the acetone method.*

PROTHROMBIN	$CaCl_2$	INCUBATION	OXALATED PLASMA	COAGULATION TIME IN MINUTES		
				Normal	Hemophilic 1	Hemophilic 2
5 drops	3 drops	0 min.	16 drops	15	14	13
5 drops	3 drops	1 min.	16 drops	13	12	12
5 drops	3 drops	3 min.	16 drops	11	11	12
5 drops	3 drops	5 min.	16 drops	8	9	10
5 drops	3 drops	7 min.	16 drops	12	9	11
5 drops	3 drops	10 min.	16 drops	14	11	13
5 drops	3 drops	15 min.	16 drops	13	15	15
5 drops	3 drops	20 min.	16 drops	12	14	15

2. *Prothrombin prepared by the heating method.*

PROTHROMBIN	$CaCl_2$	INCUBATION	OXALATED PLASMA	COAGULATION TIME IN MINUTES		
				Normal	Hemophilic 1	Hemophilic 2
10 drops	5 drops	0 min.	18 drops	12	12	12
10 drops	5 drops	1 min.	18 drops	10	10	10
10 drops	5 drops	3 min.	18 drops	13	13	15
10 drops	5 drops	5 min.	18 drops	11	10	9
10 drops	5 drops	7 min.	18 drops	9	10	10
10 drops	5 drops	10 min.	18 drops	16	12	13
10 drops	5 drops	15 min.	18 drops	11	12	12
10 drops	5 drops	20 min.	18 drops	10	13	11
10 drops	5 drops	30 min.	18 drops	15	14	14

3. *Prothrombin prepared by Gratia's method.*

PROTHROMBIN	$CaCl_2$	INCUBATION	OXALATED PLASMA	COAGULATION TIME IN MINUTES		
				Normal	Hemophilic 1	Hemophilic 2
5 drops	5 drops	0 min.	20 drops	15	16	18
5 drops	5 drops	1 min.	20 drops	13	12	15
5 drops	5 drops	3 min.	20 drops	18	13	12
5 drops	5 drops	5 min.	20 drops	9	10	11
5 drops	5 drops	7 min.	20 drops	13	9	11
5 drops	5 drops	10 min.	20 drops	12	13	13
5 drops	5 drops	15 min.	20 drops	13	13	13
5 drops	5 drops	20 min.	20 drops	15	16	16

Using the same solutions of prothrombin an experiment was made in which calcium in the prothrombin-calcium chloride mixture was precipitated by excess of oxalate at the end of each incubation period and the thrombic action was then tested on a fibrinogen solution.

PROTHROMBIN	$CaCl_2$	INCUBATION	FIBRINOGEN	COAGULATION TIME IN MINUTES		
				Normal	Hemophilic 1	Hemophilic 2
5 drops	3 drops	0 min.	8 drops	68	68	68
5 drops	3 drops	1 min.	8 drops	64	64	64
5 drops	3 drops	3 min.	8 drops	62	62	62
5 drops	3 drops	7 min.	8 drops	58	58	67
5 drops	3 drops	15 min.	8 drops	50	50	70
5 drops	3 drops	35 min.	8 drops	45	45	70

The conclusion to be drawn from these experiments is that the prothrombin of hemophilic blood does not differ in any essential particular from that of normal blood.

THE BLOOD-PLATELETS IN HEMOPHILIC BLOOD. The foregoing experiments justify the conclusion that the prothrombin in hemophilic blood does not differ noticeably in either quality or quantity from that found in normal blood. Since the other factors of coagulation, the fibrinogen, the calcium and the anticoagulants which exist in the circulating blood have been shown to be normal it is evident that the cause of the delayed clotting must be sought elsewhere. The authors believe that the clue to the right explanation is to be found in the properties of the blood-platelets of the hemophilic blood. The relative stability of these elements in hemophilic blood when shed is so striking that it must have been noted by all workers in the subject, but apparently it has been studied with care only by Minot and Lee whose work led them to the conclusion that in hemophilia there is a hereditary defect in the platelets of such a character as to make them less available for the purposes of coagulation. When a specimen of hemophilic blood is taken from a vein, without admixture of tissue extract, and placed in a glass vessel it is easy to demonstrate the presence of platelets for hours, in fact up to the time that coagulation takes place. For the purpose of comparing the behavior of the platelets in the shed blood of the normal and the hemophilic individual two series of observations were made. In the first series specimens of normal and hemophilic blood were taken from the vein and placed at once in clean vials, about 2 cc. in each vial. From time to time smears were made in the usual way up to the time of coagulation. The smears were dried, stained with Jenner's stain and examined under the oil immersion for the relative numbers of platelets existing singly or in groups. In the second series specimens of blood were taken in the same way by venepuncture, and at certain short intervals counts were made in the counting chamber of the numbers of platelets, counting only those that were single and not agglutinated into groups. The platelets were stained either by Wright's method (17) or by that of Rees and Ecker (18). The latter method gave the more satisfactory counts.

EXAMINATION OF THE SMEARS. The difference in number and appearance of the platelets as between normal and hemophilic blood was very striking. If the specimen of normal blood was placed in a paraffined vial it might remain unclotted for as long as 50 minutes. Smears made at intervals of a minute showed at first many single platelets that stained normally, but after 30 minutes very few single platelets could be found. Groups of agglutinated platelets might be found in which the individual elements showed more or less distinct signs of disintegration. Specimens of hemophilic blood taken in the same way and placed in paraffined vials remained unclotted, in our cases, for about five hours and smears taken during this time showed normal platelets although in decreasing number until shortly before coagulation set in.

The contrast in this respect between the two bloods was brought out more clearly when the specimens were placed in unparaffined vials. The normal blood under these conditions, that is, 2 cc. in glass vials having a diameter of 15 mm., clotted in from 7 to 10 minutes. Smears made at intervals of one-half minute showed the presence of single platelets, in diminishing numbers, up to 3 or 4 minutes. The agglutinated groups of platelets were conspicuous in the first smear made one-half minute after the specimen was emptied from the syringe. The appearance of the individual platelets in these groups altered rapidly. At first the hyaline border of each plate could be made out together with the more deeply stained central granules. Later the hyaline border disappeared and the central granules took on a deeper stain. Just before coagulation took place the grouped masses of platelets underwent disintegration leaving only a few bits of debris here and there. The specimens of hemophilic blood under the same conditions clotted in about 2 hours. In the first few minutes the smears showed many single plates or groups of two or three. Later larger groups occurred and the single plates became less numerous although still to be found in the smears up to 100 minutes or longer. The staining of the platelets in the agglutinated groups showed changes similar to those described above for normal blood, but these changes developed much more slowly, and only toward the end, from 100 to 120 minutes, were there obvious indications of disintegration in the massed platelets.

The prolonged survival of the platelets in the hemophilic blood was brought out clearly in the actual counts made as is shown by the figures obtained in two cases. The figures give the number of single platelets.

Experiment I

Immediately after bleeding	326000 per c.m m.
35 min. after bleeding	280000 per c.m.m.
58 min. after bleeding	248000 per c.m.m.
1 hr. 38 min. after bleeding	220000 per c.m.m.
1 hr. 53 min. after bleeding	83200 per c.m m.
2 hr. 8 min. after bleeding	36000 per c.m.m.
2 hr. 7 min. (2d count)	24000 per c.m m.

Experiment II

Immediately after bleeding	348000 per c.m.m.
5 min. after bleeding	320000 per c.m.m.
20 min. after bleeding	308000 per c.m.m.
35 min. after bleeding	200000 per c.m.m.
55 min. after bleeding	200000 per c.m.m.
1 hr. 20 min. after bleeding	195000 per c.m.m.
1 hr. 39 min. after bleeding	81000 per c.m.m.
1 hr. 47 min. after bleeding	80000 per c.m.m.
1 hr. 54 min. after bleeding	65200 per c.m.m.
1 hr. 59 min. after bleeding	14600 per c.m.m.

The first decrease in number of the separate platelets is due mainly no doubt to their agglutination into clumps as these clumps begin to appear after about 50 minutes under the conditions of the experiment and increase in number thereafter. But the marked drop just before the final coagulation is accompanied by distinct signs of disintegration in the clumped masses. It is evident that in the hemophilic blood the agglutination and disintegration of the platelets proceeds much more slowly than in normal blood under the same conditions, and it seems probable that this greater stability of the hemophilic platelets is the immediate cause of the prolongation of the clotting time. The argument may be stated in this way. In the circulating blood the plasma is incoagulable. After shedding in such way as to prevent contact with tissue extract changes take place that make the plasma coagulable. The only change definitely known to occur is the agglutination and disintegration of the platelets and since the appearance of a clot accompanies or follows upon the process of disintegration it is reasonable to assume that it is the material liberated to the plasma by the dissolution of the platelets which is responsible for initiating the process of clotting. In the blood of vertebrates lower than the mammal, in which platelets do not occur, clotting does not take place in shed blood, if care is taken to prevent contact with tissues, the plasma remains incoagulable as in the circulating blood. The causes that lead to the agglutination and disintegration of the platelets are obscure. Presumably the process begins in those platelets that come into contact with a foreign surface and possibly the material liberated to the plasma by them induces a similar process in the rest of the platelets. The material set free by the dissolution of the platelets is probably similar to that yielded by other tissue cells, that is, a lipoid material, a cephalin protein compound in which cephalin is the active material. When solutions or suspensions of cephalin are added to blood, hemophilic or normal, they hasten the clotting and the process of agglutination of the platelets. In the shed hemophilic blood, protected from tissue contact, the greater stability of the platelets slows the whole process and accounts for the delay in coagulation.

Owing to this abnormal stability of the hemophilic platelets it is possible in oxalated hemophilic blood to centrifugalize them off more completely than in normal blood and thus obtain a plasma which clots with extreme slowness on recalcification. Minot and Lee record that in an experiment of this kind it was found that the recalcified plasma did not clot until after 12 hours. In a similar experiment which we made we were successful in obtaining a plasma which would not clot at all upon recalcification. The experiment was carried out as follows. Blood was taken by venepuncture from one of our hemophilic patients whose usual clotting time by our method was round 3 hours. Some of this blood was oxalated in

the usual way in a centrifugal tube and centrifuged for 30 minutes. Specimens of the supernatant plasma were recalcified and it was found that with the optimum amount of calcium the clotting time was 3 hours.

A portion of the blood as it flowed from the vein was received into a small paraffined tube containing oxalate and packed in ice and this was centrifugalized for 2 hours with a repacking of ice at intervals of 20 minutes. At the end of this time specimens of the oxalated plasma from the upper layer, when recalcified with an optimum amount of calcium required 17 hours for clotting. The rest of the supernatant plasma was again placed in the centrifuge and centrifugalized again for two hours, without ice packing. The plasma from the upper layer of this specimen when recalcified with an optimum amount of calcium gave no clot after three days. The condition of this plasma must have been approximately identical with that of the circulating blood. It contained abundance of prothrombin as was demonstrated by preparing prothrombin from a portion by means of the acetone method. Excellent preparations were obtained which on the addition of calcium caused the prompt clotting of fibrinogen solutions.

In the circulating plasma, therefore, we have both prothrombin and calcium, but the activating effect of the latter is prevented in some way. Opinions differ on this point. According to Howell's theory it is the presence of an inhibitory substance (heparin) which prevents the activation from occurring. But whatever may be the true explanation of the normal fluidity of the blood it seems evident, and most authors agree as to this fact, that what we call intrinsic coagulation is initiated by and is immediately dependent upon the liberation of thromboplastic material from the disintegrating platelets. In hemophilic blood the platelets are abnormally stable, their disintegration takes place more slowly, hence the delay in clotting.

Some authors have assumed on indirect evidence that the thromboplastic material of the platelets is different from that found in other tissue cells. In the latter, experimenters agree in general that it is a lipoid (phosphatid) substance. According to Howell it is a protein cephalin compound, the active element being the cephalin. It would seem probable that the same substance occurs in the platelets, since they have a cytoplasmic origin from the megacaryocytes of the bone marrow. That the hemophilic platelets contain thromboplastic material of a kind similar to that found in normal platelets is shown by the fact that when obtained in suspension or solution they exhibit the same action as normal platelets in hastening the coagulation of recalcified oxalated normal plasma (Feissly and Fried, Minot and Lee). It is possible, however, that in the hemophilic platelets this thromboplastic material differs in some respect from that found in normal platelets, being a less efficient compound. Some

structural difference of this character must be assumed, in fact, to account for the greater stability of the hemophilic platelets toward the contact effect of foreign surfaces. The fact that suspensions of hemophilic platelets are less effective than those of normal platelets in hastening the coagulation of recalcified oxalated hemophilic plasma may be due to a difference of this kind in the composition of the platelets, rather than to any peculiarity of the hemophilic plasma, as has usually been thought.

CONCLUSIONS

The results are given of a study of three cases of hemophilia with special reference to the prothrombin of the blood and the characteristics of the blood-platelets.

1. The prothrombin in hemophilic blood does not differ from that of normal blood either in its concentration or its properties.

2. The blood-platelets in hemophilic blood are more resistant than those of normal blood, as shown by counting and by blood-smears in the shed blood. Their slow disintegration accounts for the delayed clotting.

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THE PREPARATION AND PROPERTIES OF PROTHROMBIN

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Notwithstanding the large literature on coagulation, only a small amount of work is reported dealing with the isolation, purification and properties of the different factors concerned in the blood clot formation. This is especially true of prothrombin.

This research has been carried on under the direction of Dr. W. H. Howell, and its purpose was to investigate the properties of prothrombin, especially the process of its activation.

Prothrombin (proserozyme Bordet-Delange) (1) is present in the blood stream in a soluble and inactive form. It is kept inactive, according to Howell (2), because of the presence of an anticoagulant (heparin), which combines with the prothrombin and prevents its activation. When bleeding occurs, the heparin is, in turn, neutralized by thromboplastin (cephalin), which is furnished by the disintegrating blood platelets and the tissue juices. The prothrombin is then activated to thrombin by the calcium present in the blood, and it, in turn, reacts with fibrinogen to form the blood clot.

PREPARATION OF PROTHROMBIN. The methods of preparing prothrombin are few and none of them gives a pure prothrombin. The methods can be divided into two groups. In one case the prothrombin is precipitated out by some reagent, while in the other the fibrinogen is precipitated leaving the prothrombin in solution. Both methods were used in my experiments.

In all of my experiments oxalated plasma from the cat was used. The animal, while under ether anesthesia, was bled from the carotid through a paraffined cannula. The blood was received into paraffined centrifugal tubes containing a sufficient amount of a 1 per cent solution of potassium oxalate (made up in a 0.9 per cent solution of sodium chloride) to give a final mixture of 0.1 per cent of the oxalate. In most cases the prothrombin was then prepared by Howell's (3) acetone method.

Oxalated plasma is taken in small quantities, preferably 5 cc., and an equal volume of acetone is added. A precipitate results which is quickly filtered through a suction filter and then washed with ether. This should be done quickly. The precipitate is spread in a thin layer on the filter

paper and dried in a current of air. The papers, when dry, are placed in a desiccator, where they will keep indefinitely without any deterioration and can be used whenever necessary. To get a prothrombin solution a paper is cut into strips and placed in 10 cc. of distilled water to which two drops of 0.5 per cent solution of sodium bicarbonate have been added. The prothrombin will all be dissolved in thirty to sixty minutes, after which the solution is filtered and the filtrate is then used. The solutions should be prepared fresh when needed, as they do not keep well for more than a day. Prothrombin prepared in this manner, on the admixture of three drops of the prothrombin solution, three drops of 0.5 per cent calcium chloride solution, and eight drops of fibrinogen, will give a solid clot in six to fourteen minutes. Prothrombin solutions obtained by the above method do not give a clot upon the admixture of the solution and calcium chloride alone. In testing the prothrombin solutions the calcium chloride and prothrombin solutions were mixed and left standing for five to ten minutes before the fibrinogen was added and then the clotting time was noted. A true clot was accepted as one where the vial could be inverted without the loss of the clot, otherwise clots were noted as sliding jelly clots or membranous clots.

Another method of preparing prothrombin was to remove the fibrinogen from the plasma by heat precipitation. In this method the oxalated plasma is heated in a water bath to from 55° to 56°C. Care should be taken not to get the temperature over 56°C. as the prothrombin is affected at higher temperatures and its activity is lessened. At a temperature of 55° to 56°C. the fibrinogen is precipitated and can be centrifuged off. This method gives an excellent prothrombin solution except for the presence of the oxalate, which, when present in excess, has an inhibitory action on the activation of prothrombin (Kuwashima) (4).

Gratia's method was also tried (5). His method consists in inoculating oxalated plasma with cultures of staphylococci. In his work he gives no indication as to the strain of staphylococci to be used. It was found, in my experiments, that a fairly fresh and virulent strain of staphylococcus aureus was the best. The plasma is inoculated, placed in an incubator at 37°C. and is kept there for six to twelve hours. In that time the fibrinogen is coagulated, forming a sliding jelly clot, and the plasma becomes opalescent. That the action of the bacteria is directly upon the fibrinogen is probable for there was never any evidence of thrombin present after defibrinating the plasma. Gratia (6) says that the fibrinogen is coagulated by a specific action of the staphylococcus, though he later seems to be undecided, stating in some reports (7) that the bacteria act as a thrombin and in others (8) that they act on proserozyme and thus coagulate the fibrinogen. The real mechanism of the bacterial action is not certain. There occurs, however, a marked lowering of the pH of the plasma during

the incubation. Before inoculation with bacteria the pH of the oxalated plasma was 7.1 and after the coagulation of the fibrinogen it was found to be 6.0. Similar results in plasma can be obtained by using hydrochloric acid in lowering the pH to 6.0, although in this case the fibrinogen is not coagulated but is precipitated. When plasma, inoculated with staphylococcus, is incubated longer than the time necessary for the coagulation of the fibrinogen, the clot is destroyed and the fibrinogen is precipitated to the bottom of the tube. The coagulation may be due to the extremely slow addition of the formed acid to the plasma. This method, though essentially the same as the heating method, in that the fibrinogen is precipitated out, is not so reliable as the latter method because of the length of time necessary to precipitate the fibrinogen, and also because one has to be sure that all the fibrinogen is gotten rid of; otherwise, a clot will result upon the addition of the calcium chloride solution to the prothrombin solution thus obtained. The incubation period must not be too prolonged as in that case the pH is lowered still more and other proteins present in the plasma are precipitated out.

In the following experiments the acetone method of preparing prothrombin was used as a rule. Wherever the other methods were resorted to, the fact is stated specifically.

The fibrinogen used in the following experiments was prepared according to Howell's (9) method, which was found to be very satisfactory. Oxalated plasma is obtained and to it is added a cold saturated solution of ammonium sulphate in the proportion of three parts of plasma to one of the sulphate solution. Centrifuge, decant the supernatant fluid, and lay over the precipitate a mixture of three parts of water and one part of the sulphate solution. Allow this to stand about five minutes and decant. Dissolve the precipitate in an amount of 1 per cent sodium chloride solution equal to the original volume of the plasma and filter. With this filtrate repeat the above process twice, making in all three precipitations. The last precipitate may not dissolve easily, in which case a few drops of 0.5 per cent sodium bicarbonate are added, a drop at a time, until the precipitate dissolves. The final solution should not give a clot spontaneously nor upon the addition of calcium chloride. The solution may be kept without deterioration for from one to five days if kept in the cold, and longer if passed through a Berkefeld filter and kept in sterile tubes, although, even if treated in this last manner, it will not keep indefinitely. It is best to prepare fresh fibrinogen whenever needed. Instead of using fibrinogen solutions one can also use the oxalated plasma.

A 0.5 per cent calcium chloride solution was used in all of my experiments.

THE ACTIVATION OF PROTHROMBIN. Prothrombin is activated to thrombin in the presence of calcium ions. The nature of this reaction is un-

known, and in the hope of throwing some light upon it, an attempt was made to determine whether it can be effected in other ways. Howell (10), as well as Minot (11) and also Nolf (12), has called attention to the fact that chloroform added to oxalated plasma may cause the formation of a clot, and this effect has been explained by Minot on the supposition that the chloroform destroys the antithrombin and thus allows thrombin, that is present in the plasma, to react with the fibrinogen.

The effects of the following reagents upon prothrombin were tested in regard to their ability to cause activation, namely, methyl, ethyl, propyl and isobutyl alcohols, chloroform, ether, acetone and carbon tetrachloride. Positive results were obtained from the action of ethyl alcohol (Bergman) (14), chloroform and carbon tetrachloride.

The effect of ethyl alcohol: A prothrombin paper was immersed in ethyl alcohol and kept in the alcohol at 40°C. for an hour. The alcohol was then poured off and the paper was dried in a current of air. When dry, the paper was cut into strips and placed in 10 cc. of water, to which two drops of 0.5 per cent solution of sodium bicarbonate had been added. After thirty minutes the solution was tested as A, while a solution of untreated prothrombin was tested as a control B.

Three drops A + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot over night
Three drops A + 8 drops fibrinogen = clot over night
Three drops B + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in six minutes
Three drops B + 8 drops fibrinogen = no clot in twenty-four hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours

Next, a prothrombin paper was cut into two equal parts. One half of the paper was returned to the desiccator, while the other half was immersed in ethyl alcohol and was kept in the alcohol at room temperature for six days. At the end of that time the alcohol was poured off and the filter paper was dried in a current of air. Then both halves of the paper, the alcohol treated A and the untreated half B, were each dissolved in 5 cc. of distilled water, filtered after thirty minutes and the filtrates tested.

Three drops A + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in ten minutes
Three drops A + 8 drops fibrinogen = clot in fifteen minutes
Three drops B + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in ten minutes
Three drops B + 8 drops fibrinogen = no clot in twenty-four hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours

Ethyl alcohol was next substituted directly for calcium chloride and the following result was obtained:

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in six minutes

Three drops prothrombin + 3 drops C_2H_5OH + 8 drops fibrinogen = clot in twelve minutes
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours

The effect of chloroform on prothrombin: The previous experiment was repeated using chloroform instead of the ethyl alcohol. A prothrombin paper was cut in two. One half of the paper was kept in the desiccator while the other half was immersed in chloroform and kept there, at room temperature, for six days. Then the excess of chloroform was poured off and the paper was dried in a current of air. Then both the chloroform treated half of the paper A and the untreated half of the paper B were dissolved, the solution filtered, and the filtrates tested.

Three drops A + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in four minutes
Three drops A + 8 drops fibrinogen = clot in four minutes
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours
Three drops B + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in six minutes
Three drops B + 8 drops fibrinogen = no clot in twenty-four hours

Chloroform was then substituted for calcium chloride in the activation of the prothrombin and the following results were noted:

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in five minutes
Three drops prothrombin + 3 drops $CHCl_3$ + 8 drops fibrinogen = clot in four minutes
Three drops prothrombin + 8 drops fibrinogen = no clot in twenty-four hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours

The effect of carbon tetrachloride on prothrombin: When carbon tetrachloride was used the same results as above were obtained. That is, prothrombin was activated to thrombin with the exception that it did not clot fibrinogen in the short time that the prothrombin activated by the other two reagents did. For instance, when carbon tetrachloride was directly substituted for calcium chloride, the following result was noted:

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in six minutes
Three drops prothrombin + 3 drops CCl_4 + 8 drops fibrinogen = clot over night
Three drops prothrombin + 8 drops fibrinogen = no clot in twenty-four hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in twenty-four hours

In these experiments fibrinogen was not coagulated when the reagents alone were added to the solution of the fibrinogen.

It is apparent from these experiments that ethyl alcohol, chloroform and carbon tetrachloride are all able to activate prothrombin. Minot, as stated before, thought that the chloroform action could be explained on the

supposition that it neutralized the antithrombin, thus permitting any thrombin present to coagulate the fibrinogen. Since, however, antithrombin is absent from the solutions of prothrombin prepared by the acetone method, it is more probable that chloroform, as well as the other two reagents, act directly on prothrombin and activate it to thrombin. The nature of the action of these reagents on prothrombin is unknown. Later experiments will show that the change from an inactive prothrombin to an active thrombin takes place spontaneously in the presence of water. Whatever may be the nature of this reaction, it is evident that the activation of the prothrombin is facilitated not only by the calcium ions but by such diverse reagents as chloroform, alcohol and carbon tetrachloride.

The other reagents used in an attempt to activate prothrombin were ether, methyl, iso-propyl and butyl alcohols. Prolonged action of ether prevents the subsequent activation of prothrombin, and when substituted directly for calcium chloride, in testing prothrombin, it causes the formation of a precipitate on addition of fibrinogen. Methyl alcohol, according to Bergman (13), may be substituted for calcium chloride. I was unable to substantiate this point, for methyl as well as butyl and isopropyl alcohols failed to cause activation. Methyl alcohol, in fact, has a destructive effect upon prothrombin, so that subsequent treatment with calcium gives no activation.

THE ACTION OF ULTRAVIOLET LIGHT ON THROMBIN AND PROTHROMBIN. In these experiments solutions of prothrombin and thrombin in quartz tubes were exposed at a fixed distance to a mercury vapor arc lamp for different periods of time. The prothrombin solutions were prepared by the acetone method. The thrombin was prepared according to the acetone method described by Howell and was used in an aqueous solution.

Unradiated prothrombin solution

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 6 minutes

Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours

Prothrombin radiated for 10 minutes

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 1½ hours

Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours

Prothrombin radiated for 20 minutes

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours

Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours

Prothrombin radiated for 30 minutes

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2½ hours

Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours

Similar experiments were made with a thrombin solution.

Unradiated thrombin

Three drops thrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 4 minutes

Three drops thrombin + 8 drops fibrinogen = clot in 4 minutes

Thrombin radiated for 10 minutes

Three drops thrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours

Three drops thrombin + 8 drops fibrinogen = clot in 2 hours

Thrombin radiated for 20 minutes

Three drops thrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours

Three drops thrombin + 8 drops fibrinogen = clot over night

Thrombin radiated 30 minutes

Three drops thrombin + 3 drops of $CaCl_2$ + 8 drops fibrinogen = clot in $2\frac{1}{2}$ hours

Three drops thrombin + 8 drops fibrinogen = no clot in 24 hours

It would seem from these experiments that thrombin is destroyed by the action of the ultraviolet light after a radiation of thirty minutes. There is left, however, some prothrombin; that is to say, the solution still causes coagulation of fibrinogen when calcium is added. It is possible that the ultraviolet light may have caused an inactivation of the thrombin or, on the other hand, it may be that the preparation of thrombin contained some prothrombin whose action became apparent after the thrombin was destroyed. It is evident that prothrombin is more resistant to ultraviolet light than the thrombin, since a radiation of thirty minutes resulted only in weakening its action.

FRACTIONAL PRECIPITATION OF PROTHROMBIN WITH AMMONIUM SULPHATE. It is well known that prothrombin belongs to the globulin group of proteins. An effort was made to determine by fractional precipitation, with ammonium sulphate solution, whether it falls into the pseudo-globulin or the euglobulin group.

In the first experiment a solution of prothrombin, prepared by the acetone method, was treated with successive additions of a cold saturated solution of ammonium sulphate so as to give mixtures in the proportion by volume of one to five, one to four, one to three, one to two, one to one, and two to one. After each addition of the ammonium sulphate, the resulting precipitate was centrifuged off and tested for the presence of prothrombin. The supernatant liquid was then carried to the next stage of precipitation.

Results: 1. Five volumes of prothrombin solution plus one volume of ammonium sulphate solution. Precipitate scanty; tested as follows:

Three drops of solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

With this concentration no prothrombin was precipitated.

2. Four volumes of prothrombin solution plus one volume of saturated ammonium sulphate. Precipitate a little larger than in previous test.

Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours

There was a slight amount of prothrombin present in the precipitate. In all probability, the prothrombin was adsorbed from the solution by the precipitate.

3. Three volumes of prothrombin solution plus one volume of the ammonium sulphate solution. Precipitate was dissolved and tested.

Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours

4. Two volumes of prothrombin solution plus one volume of ammonium sulphate. Precipitate was centrifuged off and tested.

Three drops of solution of prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 1 hour

5. One volume of prothrombin plus one volume of ammonium sulphate solution. Precipitate fairly abundant, more so than in previous precipitation, was centrifuged off, dissolved and tested.

Three drops of solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 20 minutes

6. One volume of the prothrombin solution plus two volumes of ammonium sulphate solution. Precipitate fine and granular and when dissolved and tested, reacted as follows:

Three drops of solution of this precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

The results of this experiment show that prothrombin is precipitated by saturated ammonium sulphate in concentrations of from 35 to 50 per cent and that at the latter concentration the precipitation is complete. Thus, according to the method of fractional precipitation, prothrombin belongs to the group of globulins known as pseudoglobulins. Since after saturation to 50 per cent a large amount of protein still remained in solution, this method gave a means of partial purification of the prothrombin solution which was utilized in the succeeding experiments.

SPONTANEOUS ACTIVATION OF PROTHROMBIN IN AQUEOUS SOLUTION. Prothrombin solutions were prepared by the acetone method and then precipitated by adding an equal volume of a saturated solution of ammonium sulphate. The precipitate was centrifugalized off, washed, dissolved in water and filtered. This solution was then submitted to

dialysis in a collodion sac. Dialysis was made against running water for fifteen hours and then against distilled water for twenty-four hours. Control experiments showed that none of the prothrombin passed through the dialyzing membrane. The solution in the dialyzing tube gave a precipitate. At the end of the dialysis the contents were emptied into a centrifugal tube and centrifugalized. Both the supernatant liquid and the precipitate were examined for the presence of prothrombin and thrombin. The precipitate was completely dissolved by treatment with 1 to 2 per cent solutions of sodium chloride.

Supernatant fluid

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 10 minutes

Three drops supernatant fluid + 8 drops fibrinogen = clot in 10 minutes

Dialysed precipitate

Three drops $CaCl_2$ + 8 drops fibrinogen = clot in 3 minutes

Three drops solution of precipitate + 8 drops fibrinogen = clot in 3 minutes

In the act of dialyzing, therefore, the prothrombin, in part at least, had become activated to thrombin which was found in part in the solution and in part in the precipitate. The two thrombins show one interesting difference in their action. That remaining in solution gave a permanent clot with fibrinogen, a clot which was unaffected after standing for twenty-four hours. But the thrombin found in the precipitate gave a clot with fibrinogen which redissolved in a few hours. The solution of the redissolved clot was tested with active thrombin to see if fibrinogen was present, but the result was entirely negative even when cephalin was added to the solution. Evidently, all the fibrinogen had been converted to fibrin which subsequently underwent lysis. The solution had a pH of 6.6 and when heated in a water bath gave an opalescence at 54° to 55°C., and a precipitate at 61° to 62°C.

As it seemed possible that in the long dialysis the solution would become infected and that the activation of prothrombin may have been due to bacterial action, similar experiments were carried out in which the prothrombin solution, after precipitation with 50 per cent ammonium sulphate, was dialysed under toluene, first against running water for 10 hours and then against distilled water, with frequent changes for four days. In every case a precipitate resulted and when this was separated off by centrifugalizing and both it and the supernatant liquid were tested, the same result was obtained as in the previous experiment.

Supernatant fluid

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 29 minutes

Three drops supernatant fluid + 8 drops fibrinogen = clot in 28 minutes

Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

Dissolved precipitate

Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in $4\frac{1}{2}$ minutes

Three drops solution of precipitate + 8 drops fibrinogen = clot in 4 minutes

The clots in this latter test were redissolved in four to five hours.

To determine whether the preliminary precipitation by ammonium sulphate was concerned in the activation of the prothrombin and its subsequent separation into two forms of thrombin, a dialysis experiment was carried out in which the original solution of the prothrombin was dialyzed under toluene for the times given above without previous precipitation with ammonium sulphate. The result was exactly the same.

Supernatant liquid

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 28 minutes

Three drops supernatant fluid + 8 drops fibrinogen = clot in 28 minutes

Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

Dissolved precipitate

Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 3 minutes

Three drops solution of dissolved precipitate + 8 drops fibrinogen = clot in 3 minutes

These clots redissolved in four hours.

Finally, to eliminate the possibility that the dialysis itself played some rôle in this reaction, a prothrombin solution was prepared and without precipitation with ammonium sulphate, was divided into two portions. One, A, was simply corked up and allowed to stand for four days; the other, B, was covered with toluene and kept for the same time. In both tubes a precipitate occurred which was centrifugalized off and it and the supernatant liquid were examined for thrombin. The following results were obtained:

Test of the prothrombin solution freshly made

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 4 minutes

Three drops of prothrombin + 8 drops fibrinogen = no clot in 24 hours

Tests after standing for four days:

Supernatant fluid of A

Three drops A + 3 drops $CaCl_2$ + 8 drops of fibrinogen = clot in 21 minutes

Three drops A + 8 drops of fibrinogen = clot in 21 minutes

Dissolved precipitate of A

Three drops A + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 5 minutes

Three drops A + 8 drops fibrinogen = clot in 5 minutes

Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

These latter clots were found to be redissolved over night.

Supernatant liquid B
Three drops B + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 15 minutes
Three drops B + 8 drops fibrinogen = clot in 15 minutes
Dissolved precipitate of B
Three drops B + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 10 minutes
Three drops B + 8 drops fibrinogen = clot in 8 minutes
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours
These latter clots were redissolved over night.

These experiments give clear proof that aqueous solutions of prothrombin, calcium free, undergo on standing a spontaneous activation, with the formation of two forms of thrombin, one of which is water soluble and gives a permanent clot with fibrinogen, while the other goes into solution only in the presence of electrolytes and gives a clot with fibrinogen that rapidly undergoes fibrinolysis. Experience with prothrombin prepared from the blood of different animals, indicates that the ease with which this spontaneous activation takes place varies. In the cat's blood, which has been used entirely in these experiments, the prothrombin is relatively stable, but in some other bloods, as in pig's, the activation of prothrombin occurs much more readily. Two general conclusions are suggested by these results: First, in the normal activation of prothrombin by calcium it is obvious that calcium does not form an essential constituent of the thrombin produced, since an equally active thrombin is formed in its absence. Evidence will be given later to show that calcium does not function as a catalytic agent, but that its effect is immediate. A possibility suggested by the results given above is that the calcium reacts with some portion of the prothrombin molecule or complex in such a way as to liberate thrombin, and that a similar splitting may be caused by alcohol, chloroform and other activating agents.

Second, the results obtained with the second form or the water insoluble thrombin, have a bearing on the explanation of those interesting cases met with occasionally in the literature, in which the blood after death clots promptly, but subsequently, in a few hours, undergoes fibrinolysis. In these cases the blood at autopsy may be found entirely liquid and this fact leads sometimes to the erroneous conclusion that the blood is incoagulable. As a matter of fact, the blood in such cases has clotted and the clot has redissolved. Several authors, in considering these cases, have suggested that the fibrinolysis is due to a specific fibrinolytic enzyme (Nolf) (19), but the results given above indicate that the fibrinolysis is due to the formation of a modified thrombin, of such a character that the gel formed from fibrinogen under its influence is not permanent but undergoes a slow dissolution or disintegration.

EFFECT OF CHANGING THE PH OF PROTHROMBIN SOLUTIONS. As prothrombin is a protein and may therefore be precipitated at its isoelectric

point, experiments were carried out to determine the effect of variations in the reaction of its solutions. The reagents used for varying the pH were 0.5 per cent hydrochloric acid and 0.5 per cent sodium hydroxide solutions.

A prothrombin solution was prepared by the acetone method. Its pH was found to be 6.8 and it gave a normal prothrombin reaction when tested.

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 5 minutes
Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

To the solution was added, drop by drop, some of the 0.5 per cent hydrochloric acid. After a slight addition of the acid a precipitate resulted which, when the pH of the solution was taken, was found to occur at a pH of 5.2. The precipitate was centrifuged off, dissolved in water at pH 6.8 and tested.

Three drops of solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 17 minutes
Three drops of solution of precipitate + 8 drops fibrinogen = clot in 17 minutes

The pH of the supernatant fluid was lowered by further addition of the acid but no further precipitation occurred. Then by the addition to the solution of a 0.5 per cent solution of sodium hydroxide, the pH of the solution was brought back to 6.8 and the solution was tested.

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 13 minutes
Three drops supernatant fluid + 8 drops fibrinogen = no clot in 24 hours

In varying the pH of a prothrombin solution, as in the experiments above, a precipitate was found to occur at a pH 5.2. At no other pH was I able to precipitate out any of the rest of the proteins present in the solution. The precipitate occurring at a pH 5.2 is euglobulin (Michaelis and Rona) (15). Along with this precipitate of euglobulin we get also some active thrombin. In the remaining solution prothrombin is present as shown by the above tests. This prothrombin is in fairly pure state except for the presence of the albumins. To get rid of the albumins an equal volume of saturated ammonium sulphate was added to the solution. A precipitate resulted which was centrifuged off, washed, dissolved and tested.

Three drops of solution of the precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 28 minutes
Three drops of solution of the precipitate + 8 drops fibrinogen = no clot

The solution of the above precipitate was then placed in a collodion tube and dialyzed against running water for ten hours and with frequent changes against distilled water for four days. As in the previous experiments, on dialysis of the prothrombin solution a precipitate resulted. The contents of the dialysis tube were then emptied into a centrifugal tube and centrifugalized. Both the supernatant liquid and the precipitate were examined for the presence of thrombin and prothrombin. The precipitate was washed with water and dissolved in 1 to 2 per cent solution of sodium chloride.

Supernatant liquid

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 30 minutes

Three drops supernatant fluid + 8 drops fibrinogen = clot in 30 minutes

Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

Dissolved precipitate

Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 3 minutes

Three drops solution of precipitate + 3 drops fibrinogen = clot in 3 minutes

These latter clots redissolved, as in previous experiments, in two hours

A prothrombin solution, prepared by the acetone method, gives a precipitate when brought to an acidity of pH 5.2 which contains thrombin. This thrombin was not present as such in the original solution, but was formed in consequence of the change in the reaction of the solution. Further addition of acid caused no additional precipitate nor was it possible to obtain any precipitate on varying the reaction of the solution on the alkaline side. After obtaining a precipitate at a pH 5.2, the reaction of the solution was brought to pH 6.8 and it was found to give a normal prothrombin reaction when tested. The prothrombin remaining in solution can be precipitated out by the addition of an equal volume of saturated ammonium sulphate, and when that precipitate is dissolved and dialyzed, the two forms of thrombin were obtained, one water soluble and the other water insoluble and fibrinolytic.

STABILITY OF PROTHROMBIN SOLUTION. As shown by the previous experiments, prothrombin cannot be kept in solution for any length of time without conversion into the two types of thrombin. Spontaneous activation of prothrombin in solution takes place in from twenty-four to thirty-six hours at room temperature, and faster at higher temperatures. It was thought possible that a stable form of prothrombin solution might be obtained by first passing it through a Berkefeld filter, receiving the filtrate into a sterile container and then placing the filtrate into sterile tubes and sealing them off. The experiment was carried out but altogether negative results were obtained. A prothrombin solution was prepared according to the acetone method and tested.

Three drops prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 5 minutes
Three drops prothrombin + 8 drops fibrinogen = no clot in 24 hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot

The solution was then passed, under aseptic precautions, through a Berkefeld filter and the filtrate was examined for prothrombin.

Three drops filtered prothrombin + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 7 minutes
Three drops filtered prothrombin + 8 drops fibrinogen = no clot in 24 hours

The filtrate was placed in sterile tubes and the tubes were sealed off with a flame. The solution in the tubes was at first clear. In two days a slight opalescence was noticed and in four days a precipitate settled to the bottom of the tube. The contents of one of the tubes were emptied into a centrifugal tube and centrifugalized. Both the precipitate and supernatant liquid were tested.

Supernatant liquid
Three drops supernatant liquid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 41 minutes
Three drops supernatant liquid + 8 drops fibrinogen = clot over night
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours
Dissolved precipitate
Three drops solution of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = no clot in 10 minutes
Three drops solution of precipitate + 8 drops fibrinogen = clot in 10 minutes
The clots caused by the action of the dissolved precipitate on fibrinogen dissolved in four to six hours

The rest of the tubes containing the filtered prothrombin solution were left standing for four months and were then examined for prothrombin and thrombin. At that time neither the precipitate nor the supernatnat liquid possessed any coagulative power. We can conclude, then, that it is impossible, at least with acetone prepared prothrombin, to keep a solution of prothrombin for any length of time. The same change always occurs, namely, the formation in the solution of the water soluble and the water insoluble thrombin. On long standing, even under aseptic conditions, the solution loses all of its coagulative action.

ACTIVATION OF PROTHROMBIN BY CALCIUM CHLORIDE. The rôle of calcium ions in the activation of prothrombin is accepted by all workers, but the time necessary for the reaction has not been investigated, although it seems to be assumed that it is a progressive reaction requiring a relatively long period for completion.

A prothrombin solution was prepared according to the acetone method and it was divided into five parts. To part one an equal volume of 0.5

per cent calcium chloride was added, the mixture was shaken very quickly, and to it was added an equal volume of acetone. A precipitate resulted. The contents were then emptied into a centrifugal tube and centrifuged for fifteen minutes. The precipitate was spread in a thin layer over a filter paper and dried in a current of air. When completely dried a solution was made of the precipitate, by dissolving it in water, and tested.

Three drops dissolved precipitate I + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours
Three drops dissolved precipitate I + 8 drops fibrinogen = clot in 2 hours
Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

The same experiment was repeated with parts 2, 3, 4 and 5 except that in treating part 2 of the original prothrombin solution, five minutes were allowed, after the admixture of 0.5 per cent calcium chloride and the prothrombin solution, before the acetone was added to precipitate the formed thrombin. In part 3 ten minutes were allowed, part 4 fifteen minutes, and in part 5 twenty minutes for the activation of the prothrombin by the calcium chloride solution. In all tests the precipitate was centrifugalized, spread over filter paper and dried. A water extract was obtained from these papers and they were all tested as in part one.

Dissolved precipitate of part 2
Three drops dissolved precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours
Three drops dissolved precipitate + 8 drops fibrinogen = clot in 2 hours
Dissolved precipitate of part 3
Three drops dissolved precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours
Three drops dissolved precipitate + 8 drops fibrinogen = clot in 2 hours
Dissolved precipitate of part 4
Three drops of dissolved precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours
Three drops of dissolved precipitate + 8 drops fibrinogen = clot in 2 hours
Dissolved precipitate of part 5
Three drops of dissolved precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 2 hours
Three drops of dissolved precipitate + 8 drops fibrinogen = clot in 2 hours

That the activation of the prothrombin in the above experiment was not due to the acetone, was proved by running a control. A prothrombin solution was prepared by the acetone method. An equal volume of acetone was then added to this solution and the prothrombin was reprecipitated which, when tested, caused no coagulation in a fibrinogen solution if used alone, but gave a clot in nine minutes when calcium chloride was added.

It is evident, from the above experiments, that calcium activates pro-

thrombin instantaneously. Just as good thrombin action was obtained from prothrombin that was in contact with calcium for a moment as from that exposed for twenty minutes. The same fact is brought out in another way in the following experiment.

A prothrombin solution was prepared by the acetone method. Twelve vials were next taken and into each were placed 5 drops of the solution. Then 3 drops of 0.5 per cent calcium chloride solution were added to all the vials at approximately the same time. At intervals varying from zero to thirty minutes, oxalated plasma was added and the clotting time was noted. The amount of calcium chloride was not enough to coagulate by itself the amount of oxalated plasma used.

RESULTS:

Drops of prothrombin + drops $CaCl_2$ + incubation period + oxalated plasma = clot in minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 0 minutes + 16 oxalated plasma = precipitate at bottom of tube

5 drops of prothrombin + 3 drops $CaCl_2$ + 1 minute + 16 oxalated plasma = 6 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 3 minutes + 16 oxalated plasma = 4 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 5 minutes + 16 oxalated plasma = 2 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 7 minutes + 16 oxalated plasma = 2 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 9 minutes + 16 oxalated plasma = 2 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 11 minutes + 16 oxalated plasma = 3 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 13 minutes + 16 oxalated plasma = 4 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 15 minutes + 16 oxalated plasma = 6 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 20 minutes + 16 oxalated plasma = 23 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 25 minutes + 16 oxalated plasma = 28 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 30 minutes + 16 oxalated plasma = 81 minutes

5 drops of prothrombin + 3 drops $CaCl_2$ + 30 minutes + 16 oxalated plasma = 81 minutes

This experiment indicates that the activation of prothrombin takes place immediately, although the maximum effect was obtained after an interval of several minutes. In the above experiments this interval was found to be from three to six minutes. The prolongation of the clotting time observed after a long interval of incubation with calcium can only be referred to some inhibiting influence of the calcium. It is well known that an excess of calcium causes a retardation of the clotting process and possibly

the same phenomenon occurs with smaller concentrations when the time of exposure is prolonged.

The same experiments were repeated using prothrombin solution prepared by Gratia's and by the heating method, with practically the same results. In these experiments the optimum time for the activation of the prothrombin was found to be slightly lengthened as compared to the optimum activation time of prothrombin prepared by the acetone method. This slight increase in the optimum time for the activation of the prothrombin was probably due to the presence of oxalate, which was present both in the prothrombin solution prepared by these two methods and also in the oxalated plasma used in place of fibrinogen. That oxalates, even when slightly in excess, have an inhibitory action on the process of clotting, has been demonstrated by Stuber and Sano (16), and by Kuwashima (17).

THE DIRECT ACTION OF CALCIUM ON PROTHROMBIN. In the experiments made upon spontaneous activation of prothrombin in solution, either by dialysis or by simple standing of a prothrombin solution for a certain time, it was found, as is demonstrated in the experiments, that two forms of thrombin were obtained. Of these two thrombins one is water insoluble, in which the clotting of the fibrinogen is followed by fibrinolysis, and the other is water soluble in which clots formed by its action on fibrinogen are not dissolved. The question arose as to whether similar results could be obtained by the activation of a prothrombin solution by calcium.

A prothrombin solution was prepared by the acetone method. It was centrifuged for thirty minutes and at the end of that time no sign of a precipitate was observed in the centrifugal tube. An equal volume of 0.5 per cent calcium chloride was added to the solution, the mixture was well shaken and then centrifugalized for five minutes. At the end of centrifugalization a precipitate was obtained at the bottom of the tube. Both the supernatant liquid and an extract of the precipitate, which was found to be quite insoluble even in high concentrations of sodium chloride solution, were tested.

Supernatant fluid

Three drops supernatant fluid + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 6 minutes

Three drops supernatant fluid + 8 drops fibrinogen = clot in 5 minutes

Extract of the precipitate

Three drops of the extract of precipitate + 3 drops $CaCl_2$ + 8 drops fibrinogen = clot in 45 minutes

Three drops of the extract of precipitate + 8 drops fibrinogen = clot in 45 minutes

Three drops $CaCl_2$ + 8 drops fibrinogen = no clot in 24 hours

These latter clots were redissolved in 6 to 14 hours.

It is evident that in the activation of prothrombin by calcium, as in the spontaneous activation of prothrombin in an aqueous solution, two types

of thrombin are formed. Of the two thrombins, one is water insoluble and fibrinolytic, while the other is water soluble and possesses no fibrinolytic action. When both forms are present, complete fibrinolysis does not occur, because, although the fibrin produced by the water insoluble thrombin may be dissolved, the fibrin produced by the water soluble form is not, and as a result the clot formed remains intact.

Discussion. In the preparation of prothrombin I have found the acetone method the best. It is a very simple method and gives a good prothrombin preparation that can be kept indefinitely. The plasma heating method is good except for the fact that there is a possibility of causing injury to the prothrombin present. Gratia's method is not so reliable or convenient. First, one has to be certain that the bacteria will coagulate the fibrinogen in the plasma and only the staphylococcus aureus was found, at least in my experiments, to give this reaction satisfactorily. The inoculated plasma must be incubated for at least six to twelve hours, and even then one is not certain that all the fibrinogen is coagulated. If the incubation period is too long, other plasma proteins will be precipitated out by the lowering of the pH, resulting from the action of the staphylococci. In both the heating method and Gratia's method we have oxalate present, in the prothrombin solution, which exerts some inhibiting influence upon the coagulation. According to Stuber and Sano this inhibitory action, as well as that of citrates and bile salts, is due to the formation of dissociable salts with the fibrinogen which diminish the coagulability of the fibrinogen.

In regard to the activation of prothrombin, it has been shown in these experiments that the formation of thrombin from prothrombin can be effected in a variety of ways. Namely, by the action of calcium ions, by dialysis, or spontaneously in an aqueous solution on standing, and by the action of such reagents as ethyl alcohol, chloroform and carbon tetrachloride. While the nature of this reaction is unknown, there is some basis for the belief that the change produced is essentially the same in all cases. Dialysis and the spontaneous reaction in an aqueous solution give two different forms of thrombin, one water soluble and one water insoluble, the latter showing the peculiarity that the fibrin formed by it subsequently redissolves. In the reaction with calcium two similar thrombins are formed.

The effect obtained on dialysis recalls the experimental results of Chick (18) on the dialysis of pseudoglobulin solutions. She found that on dialysis of a pseudoglobulin a precipitate formed (euglobulin) which contained phosphorus, while the water soluble globulin remaining in solution was free from phosphorus or contained it only in traces. The same results were obtained by Hardy (19) and by Haslam (20). Addition of lecithin to the latter solution, water soluble part, and renewed dialysis again

caused a precipitate (euglobulin) containing phosphorus. She makes the suggestion that euglobulin represents a compound of water soluble globulin (pseudoglobulin) with a phosphorus containing lipoid.

I have been able to corroborate these results upon my preparations. A solution of prothrombin prepared by the acetone method and tested for its activity was precipitated by the additon of an equal volume of saturated solution of ammonium sulphate. The precipitate was centrifugalized off and dissolved in a volume of water equal to that of the original prothrombin solution. This solution was dialyzed against running water for four hours, until it failed to give a reaction for sulphate. It was then divided into two equal parts, A and B. A was evaporated to dryness, giving a residue of 0.3238 gram. With this material a phosphorus determination was made by Neumann's method. B was dialyzed in a collodion tube for five days against distilled water. As in previous experiments a precipitate was formed. The contents of the dialyzing tube were emptied into a centrifugal tube and centrifugalized, giving a precipitate and a supernatant liquid. Each of these was dried to constant weight and analyzed for phosphorus by Neumann's method. The results were as follows:

Solution A:
Weight of dry material = 0.3238 gram
Weight of phosphorus = 0.0276 gram

Solution B:
Supernatant liquid—weight of dry material = 0.0807 gram
Supernatant liquid—weight of phosphorus = none
Precipitate—weight of dry material = 0.0815 gram
Precipitate—weight of phosphorus = 0.0273 gram

The supernatant liquid, after prolonged dialysis, in which, according to my previous experiments, is found the water soluble thrombin, contained no phosphorus, while the precipitate containing the modified and water insoluble form was rich in phosphorus. Whether or not the phosphorus containing material in the precipitate was combined with the modified thrombin can not be determined. In view, however, of Bordet's theory of the structure of thrombin as a compound of serozyme and cytozyme it is interesting to note that the water soluble thrombin in the supernatant liquid, which, according to its reactions with fibrinogen, is the normal form of thrombin, gave no detectable reaction for phosphorus.

The fact that prothrombin during the process of activation seems to dissociate into two portions which differ in their solubility in water, in the properties of the fibrin formed by their action on fibrinogen, and possibly in their content in phosphorus, suggests that the original prothrombin molecule or aggregate may consist of two different thrombin groupings, and that the process of activation consists in a reaction with or precipitation of one of these groups, the water insoluble (phosphorus

containing) fraction, with the liberation of the other in solution. On some such hypothesis one may understand how activation occurs with such different reagents. Further experiments are necessary to give this hypothesis an adequate experimental basis.

CONCLUSIONS

1. The acetone method of prothrombin preparation was found to be the most efficient and reliable.

2. Ethyl alcohol, chloroform and carbon tetrachloride can all cause the activation of prothrombin prepared by the acetone method.

3. Ultraviolet light destroys thrombin, while prothrombin is found to be more resistant to radiation.

4. Prothrombin is a pseudoglobulin, being precipitated completely out of solution with a concentration of ammonium sulphate of 30 to 50 per cent.

5. On dialysis or on standing in solution, prothrombin is converted into a water soluble and water insoluble thrombin. The fibrin produced by the latter variety subsequently undergoes fibrinolysis. In the activation of prothrombin by calcium, two similar forms of thrombin are produced. Sterile solutions of prothrombin, if kept for long periods, eventually lose all power of causing coagulation.

6. When a solution of prothrombin (acetone method) is acidified with hydrochloric acid, a precipitate occurs at pH 5.2 which exhibits thrombic activity. Most of the prothrombin remains in solution in inactive form.

7. The provisional hypothesis is made that the prothrombin molecule or complex consists of two thrombin groups. Activation by calcium or other reagents, or by standing in aqueous solution, consists in the precipitation of one of these groups as a modified thrombin, water insoluble and fibrinolytic—the other remains in solution as normal thrombin.

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THE FEMALE SEX CYCLE AS A FACTOR IN LEARNING IN THE RAT

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Although the age at which Hubbert (1915) found rats to learn most easily does not agree with the ages for which Slonaker (1907), (1912) reports greatest activity in rats, it seemed possible to think of the increased activity that Slonaker (1924) and Wang (1923) report during oestrum as of a "seeking" nature rather than mere increased physical fitness such as the activity during the "prime of life" might be due to. This might facilitate the learning of such a habit as maze running. To test this the following experiment was undertaken.

EXPERIMENTAL METHOD. Thirty-two young mature females were trained in the simple maze described by Lashley (1918). He has shown that this maze gives significant differences in speed of learning with difference in distribution of practice and that these differences are in accord with those obtained by Ulrich (1915) using the circular maze. It was therefore considered an adequate measure of learning ability.

The rats were examined by a slight modification of Long and Evans' (1922) method suggested by Selle's (1922) pipette procedure used with guinea pigs. Instead of using a spatula, a drop of saline was pulled in and out of the tip of a medicine dropper inserted into the vagina. It is easier to control the amount of pressure exerted on the bulb if it is pushed down over the glass tube so that the latter extends all the way back into the rubber bulb. The saline was dropped onto a slide and examined without a cover glass. Eight or 10 drops can be put onto one slide before it needs to be cleaned. This not only speeds up examinations considerably but there are always enough cells to permit a diagnosis of the stage of the cycle the animal is in. This was not always true of the spatula method as I used it. This pipette method also more completely clears the vagina of free cells at each examination so that the samples are not mixed in cases where examinations are being made every 3 or 4 hours so as to determine the beginning of the second stage.

Sixteen rats were trained during the first part of the second stage when cornified cells first appeared. Each of the other 16 was paired with one of the first group and run as a control whenever her partner was in heat. If

oestrum occurred in both members of a pair at the same time the control animal was fed at the usual time and run the following day at the same hour the partner had been run.

At first the rats were fed bread and milk for 15 minutes once a day. This diet was found to be too limited in kind and amount to permit short or regular cycles. Therefore they were later fed table scraps and allowed to eat for an hour every day. After each set of trials in the maze they were left with food for 24 hours since they would not be run again for several days. This kept them in excellent condition so that cycles were usually 4 to 6 days long and fairly regular. This is approximately what Long and Evans find to be the normal length of cycle. This regime increased the time of learning but that food was still an incentive is shown by the fact that they learned at all.

The criterion of learning was 9 out of 10 trials without error.

TABLE 1

Speed of learning the simple maze, during oestrum and dioestrum

	TRIALS				TIME (IN SECONDS)				ERRORS			
	Aver.	S.D.	Med.	Med.V.	Aver.	S.D.	Med.	Med.V.	Aver.	S.D.	Med.	Med.V.
Rats trained during oestrum.	19.3	12.0	18.0	6.0	891.8	1190.0	263.0	214.0	17.0	13.4	13.0	6.0
Rats trained during dioestrum	26.9	30.3	18.0	10.0	588.8	691.1	305.5	229.0	21.1	17.8	15.0	10.5
Difference	7.6±5.5		0		303.0±232.2		42.5		4.1±3.7		2.0	
Difference / P.E. dif.	1.38				1.30				1.11			

RESULTS. Table 1 shows the averages, standard deviations, medians and median variations of the trials, errors and time required by rats trained during oestrum and those trained during dioestrum to learn the simple maze. In the third row are the differences between the medians of the two groups and the differences between the averages with the probable errors of the latter. The fourth row contains the ratio between the difference between averages and its probable error. This figure should be 4 or more to indicate a significant difference between the two experimental groups.

Using time as a measure of learning and averages as the measure of central tendency, the control animals learn slightly more easily than the heat animals. But using trials or errors as a measure of learning, or medians except in the case of trials, the heat animals learn slightly more easily than the control animals. In no case is the difference more than 1.38 times its probable error, whereas to indicate a significant difference it should be over 4 times its probable error. In this case, then, where trials are used as

a measure of learning, there are 83 chances in 100 that a difference in the same direction will be found if the experiment is repeated. But there are only 38 chances in 100 that the difference found upon repetition will be as great as 10 and there are as many as 17 chances that the difference will actually be in the opposite direction, i.e., that the rats trained during dioestrum will learn in fewer trials than those trained during oestrum. It must also be remembered that this is the measure that shows the most significant difference and it should be noted that when the medians of these same data are used they are identical for the two groups. The averages are weighted by two extreme cases in the control group that required 74 and 111 trials respectively to learn the maze while no other rat in this group required more than 34 trials.

In view of these facts and considering that the number of cases is only 16 and the variability very great, it may be said that these data fail to indicate any significant difference between the two groups.

Discussion. It cannot be concluded from the above results that the female sex cycle is never a factor in learning, since incentives have not been controlled. Moss (1924) found that 3 out of 5 female rats in heat braved a 28 volt shock to reach a male. This is 60 per cent as compared with 1 out of 4 or 25 per cent that were willing to take this same shock to get food under conditions of starvation similar to those used in this experiment, i.e., 24 hours without food after an adequate diet had been maintained. Although this number of cases is small such results suggest that learning might be much more rapid if a sex object were used for the animals in heat since the sex object seems to be a stronger incentive than food unless the animals are much hungrier than they were in this experiment. But the control animals would probably not be attracted by the sex object.

It is also conceivable that when the value of the sex object is enhanced with the onset of oestrum, the attractive value of food becomes correspondingly less, so that food would be a greater incentive for the control animals than for those tested during oestrum. Under these conditions it would be possible to explain the lack of difference between the two groups indicated by this experiment by supposing that some such factor as the increased activity that has been observed during oestrum, facilitates learning during this period, while the decreased incentive retards performance. The two factors would thus have a neutralizing influence on each other and the result would be that their actual performance would be the same as that of the control group. However there are no indications in the behavior of the animals that warrant such an interpretation. It seems more probable that the learning process is actually unaffected by oestrum.

SUMMARY

Sixteen rats were run in the simple maze during oestrum with 16 control animals run during dioestrum. No significant difference in speed of learning was found between the two groups. It is concluded that when food is the incentive the female sex cycle is probably not an important factor in learning the maze.

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EXPERIMENTAL ANALYSIS OF THE SIMPLE ACTION POTENTIAL WAVE IN NERVE BY THE CATHODE RAY OSCILLOGRAPH

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The action potential wave that is started by the application of an induction shock to a nerve changes its form during propagation. These form changes are of two varieties, both of which, however, may be exhibited by the same nerve. This is true, for example, of the action wave in the sciatic and saphenous nerves in which there can be distinguished both a separation out of waves, and a broadening and flattening of these waves. In the phrenic nerve, on the other hand, the action wave merely broadens and flattens during propagation. The separation out of waves is due, it has been found (Erlanger, Gasser and Bishop, 1924), to group differences in the conduction rates of the constituent fibers. Action currents exhibiting these waves have been designated *compound* in contradistinction to the waves themselves and to the waveless action currents which were designated *simple* notwithstanding the obvious possibility of explaining their change in form upon the basis of slight differences in the rate of propagation of action potentials in the individual nerve fibers (Erlanger, Gasser and Bishop, 1924). As a matter of fact all of our experiences indicate that this so-called simple action potential actually is a composite wave made up of *axon potential waves* which, owing to differences in propagation rate, get out of phase as they move along the nerve from the point stimulated. In the present paper are brought together the experiments supporting this view.

APPARATUS. The apparatus employed is the same as has been described heretofore (Gasser and Erlanger, 1922), a three-stage amplifier working into a cathode ray oscillograph, the time axis of which is obtained by charging a condenser of known capacity in parallel with the vertical plates of the tube, through a known resistance. Certain modifications have been adopted for readier manipulation.

As in the past, it has been found impracticable to photograph the standing wave on the face of the tube with a lens system, while the method of making contact prints has the disadvantage, first, that the film does not

lie in close contact with the spherical end of the oscillograph tube, and second, that radiation occurs from the fluorescent screen through the thickness of glass, making a much broader line on the film than the screen presents to the eye. The first condition of poor contact aggravates the second. To secure better contact, the film ($4 \times 2\frac{1}{2}$ inches) may be perforated in the midline a quarter of the way from either end, and then slit longitudinally from either end into these holes. To hold such films in position on the tube, a ring with a thin sheet of rubber stretched over it and sliding along a rod parallel to and below the long axis of the Braun tube can be forced over the face of the tube, the stretched rubber fitting the film to the glass by causing the edges of the slits to overlap. The horizontal and vertical meridians of the tube's surface are thus brought into good contact with the film, and the events to be photographed are adjusted to these positions, where, upon development of this curved surface when the film is again flattened, the distortion due to curvature is at a minimum. Since this adjustment takes sufficient time to fog the film, and since the manipulation necessary causes vibration of the plates of the Braun tube sufficient to cause flickering of the figure to be photographed, the filament current must be reduced below the point of luminosity during adjustment. It cannot be turned off completely, because the building up again of the filament current without arcing is a slow process, and would again involve fogging the film. Three resistances are therefore put in series in the filament circuit. The first is a control rheostat for building up the current. The second is a micrometer rheostat for accurate focussing, which is accomplished by fine adjustment of the filament current. These two are set and left undisturbed. The third resistance when inserted is just sufficient to remove the tube fluorescence; when it is removed, which can be done instantaneously, it acts like a shutter to expose the film without jarring the apparatus.

The lateral adjustment of the zero of the line on the Braun tube is accomplished as heretofore by regulating the strength of a magnetic field of vertical air-core coils placed above and below the neck of the tube at the region of the deflecting plates. The vertical adjustment of the base line is brought about by interposing a 36 volt battery of small Ever-Ready dry cells between the 500,000 ohm leak from the output condenser, and the ground. This battery is tapped to switch points on the instrument board in such a way that the "zero" potential of the horizontal Braun tube plates can be "boosted" by steps upwards or downwards, plus or minus 36 volts, by setting a dial. The record of the amplified action potential then appears on this boosted line as a base line, permitting a larger deflection to one side of the base line than if this were across the middle of the tube.

The "spreader" condenser, the charging of which gives the time axis, instead of charging through the commutator during one half a revolution,

and discharging the other half, is charged directly and continuously from the battery through a resistance, and only discharged through the commutator. This eliminates the effect of one sliding contact during the record, and, with a low resistance in the discharge circuit (30 ohms), the condenser is still discharged virtually to zero potential. Since potential does not appear in the time formula, being represented by the length of the line on the face of the Braun tube, a residual potential in the condenser will introduce no error.

The moist chamber, consisting of a water jacketed thermostat, has been rearranged so as to shorten leads to and from nerve and to facilitate manipulation. From the secondary coil switches mounted on top of the

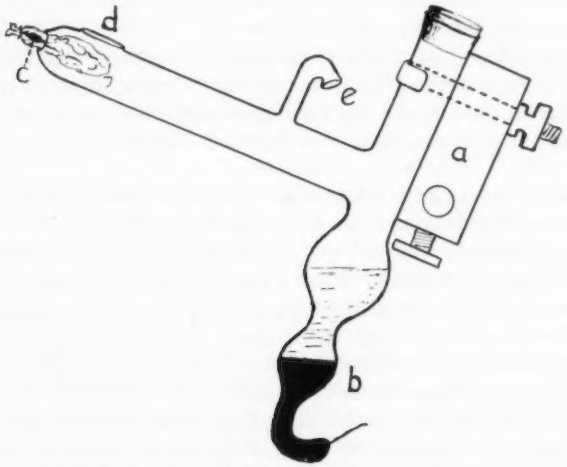

Fig. 1. Nonpolarizable calomel electrode for leading action potential from nerve. *a*, clamp; *b*, mercury-calomel filled bulb; *c*, knotted yarn, two fine strands of which are tied together around nerve; *d*, aperture for replacing yarn contact and refilling; *e*, overflow tube for washing out with Ringer's solution, from *d*.

chamber, six double point platinum stimulating electrodes are led into the interior. Six nonpolarizable calomel electrodes are mounted in the chamber, their leads passing out through an aperture at the bottom of the thermostat to switches closely adjacent to the amplifier's input. The nerve can be mounted on any or all of them in such a way as to make possible the alteration of the points on the nerve stimulated and led from in accordance with the plan of the experiment.

The nonpolarizable electrodes (fig. 1), made of Pyrex tubing, consist of a perpendicular section closed above with a rubber stopper and terminating

below in a bulb with a platinum lead, and a horizontal T-branch open at the end. This T-arm has a second opening just back from the end on the upper side of the tube, through which a moist yarn can be inserted and drawn out through the small end aperture. A second perpendicular T-branch, *e*, is fused into this horizontal part near its junction with the perpendicular stem, which facilitates filling the electrode without including air bubbles. The lower bulb is charged with mercury and commercial calomel and the rest of the electrode is filled with Ringer's solution after the nerve is in place, through the aperture near the end. These electrodes have a resistance of 1500 to 6000 ohms, depending largely upon the area of the calomel-mercury surface. They are strictly nonpolarizable with the currents used and seem to be good indefinitely without renewal, though the yarn-nerve contacts are changed before each day's operation, and the stems washed out with fresh Ringer's solution.

It has been found advisable to separate the ground leads for various parts of the apparatus. The amplifier, the primary stimulating current, and the Braun tube circuit are grounded respectively to a water pipe, a sewer pipe and a steam pipe, these grounds being from 0.3 to 0.5 ohm apiece.

In the amplifier the grid leak from the first panel has been removed (since this grid is grounded through the nerve) and the grid leak of the third panel has been decreased from 500,000 to 100,000 ohms to accommodate the amplified input of this panel, which, with a higher resistance, showed a cumulative charge on the grid with a strongly positive potential, due to electron flow from the filament to the grid. This variable grid had caused a shift in the base line with large action potentials, and a slightly different characteristic for different input values. The reduction of this leak resistance has lowered the amplification but has straightened and stabilized the characteristic of the tube working under these conditions. The third panel has a grid bias battery of -1.5 volts in series with the leak.

Work with the apparatus has indicated certain precautions to be necessary, while others heretofore observed we have been able to disregard. The input cannot be too well shielded; the primary of the induction coil must have one side grounded through not more than 50,000 ohms; the secondary capacity should be small to avoid surge effects; the primary should have sufficient series resistance (a few ohms) to cut down arcing at the break of the commutator, and sufficient voltage and consequent current through the coil to allow the secondary to be very loosely coupled with the primary, thus avoiding capacitative coupling through the coils from the amplifier input to the ground of the primary. It has been found unnecessary to shield either the A or B amplifier or Braun tube batteries or leads. The batteries are charged the day before use, and after a night's rest work at constant potential for the duration of an experiment. Under

these constant conditions it has proved feasible to plot a considerable number of the functions of the apparatus in the form of graphs, and such values as the amplitude-voltage relationships of the action potential wave, time intervals per millimeter on the Braun tube base line, etc., can be read from the curves without calculation or correction.

MATERIAL AND TECHNIQUE. In the following experiments, green frog sciatic (*Rana pipiens*), bullfrog sciatic (*Rana catesbiana*) and dog phrenic nerves have been used, the first two at room temperature in air, the latter at 37.5°C. in oxygen. The green frog sciatic has the disadvantage that the peripheral section where there are no branches is short, and cut branches not only affect slightly the conduction rate in normal nerve fibers in their vicinity, but also affect the shape of the action wave when they leave the main nerve between the leading-off electrodes. The bullfrog sciatic is open to the same objection, except that the unbranched stretch is longer, amounting to as much as 80 mm. The ideal nerve is the phrenic, and our best records were obtained from phrenics from which the connective tissue sheath had been dissected off, in whole, or at the level of the leading-off electrode.

Unless it is otherwise stated the stimuli in the case of the phrenic nerve have been maximal and in the case of the frog's sciatic nerve, just maximal for α. The action waves obtained in this way from different points on the phrenic nerve are definitely maximal and therefore comparable. In the case of the sciatic nerve, however, the margin between the maximal stimulus for α and the threshold for β, as will be seen, is narrow or absent and the finding of the just maximal α stimulus may therefore become a matter of judgment. When a comparison is to be made between the results of stimulating different points on the nerve it is necessary on account of differences in secondary resistance resulting from differences in the thickness of the nerve and in the breadth of the contact with the nerve, to adjust the strength of current to maximal for α with each shift of the connections on the nerve. Furthermore, readjustments must be made as the irritability of the nerve changes during the course of the experiment. It can be readily understood that under these circumstances the action potentials of "just maximal α waves" can be only roughly comparable one with the other. All the leads were made with the electrode to the grid of the amplifier connected to the end of the nerve which had been killed by heating.

EVIDENCE DERIVED FROM THE CHANGE IN THE FORM OF THE SIMPLE ACTION POTENTIAL DURING CONDUCTION. On the hypothesis that the simple action wave is constituted of axon potential waves travelling at different rates it is possible to construct diagrams which serve to visualize the responses one might expect to obtain experimentally. In constructing these diagrams it has been assumed that in any given nerve the axon poten-

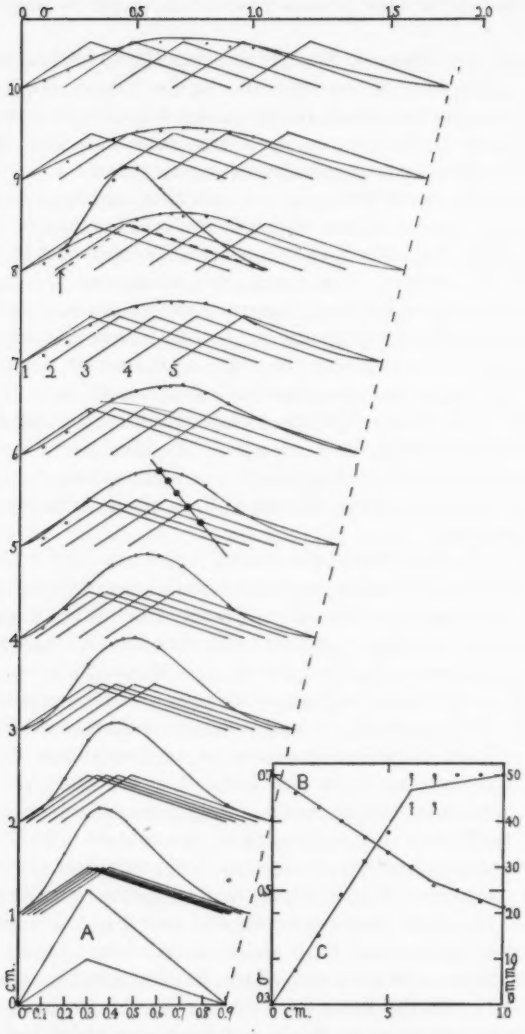

Fig. 2. *A*. Diagrammatic representation of an action potential wave based upon the assumption that it is composed of the sum of similar axon action waves conducted at different, but regularly distributed, rates. The diagrams picture five axon waves (*1*, *2*, *3*, *4* and *5*) travelling at the rates of 42, 39, 36, 33 and 30 m.p.s. respectively, and their sum (divided by 2 in order to reduce it to a convenient height) at distances from the site of stimulation of 0 to 10 cm. as indicated on the vertical axis. Abscissae, time in σ; ordinates, distance of conduction in centimeters.

B. Distance of conduction (abscissae) plotted against amplitude (ordinates) of the composite waves in *A*.

C. Distance of conduction (abscissae) plotted against the time to maximum (ordinates) of the composite waves in *A*.

tial waves are all alike in duration but travel at different though uniform rates in the individual fibers. The axon waves have been drawn as triangles, an alteration from fact which will not materially affect the inferences to be derived from the constructions. In the diagram selected for publication (fig. 2) the time to maximum of the triangles representing the axon waves in frog's nerve and the total duration are given values of 0.3 and 0.9σ respectively. These times, as will be seen, are slightly briefer than those of the shortest sciatic axon potential wave. The corresponding values chosen for the constructions representing the phrenic action potential wave have been 0.2 and 0.6σ respectively. These likewise, as will be seen later on, are slightly shorter than the actual values. The amplitude of the axon action wave triangles and the range and the distribution of their conduction rates have been varied in the various constructions and in this way it has been found that there are pertaining to this genus of constructions certain general rules to which reference will be made each in its proper place. One of the many constructions we have made is reproduced in figure 2. In this case the action of five of the many fibers composing the nerve is represented. Their potentials (heights) are here represented as alike, but they are given conduction rates ranging evenly between 42 and 30 m.p.s. This range probably approximates that obtaining in the α wave of the bullfrog's sciatic, 42 m.p.s. being a usual rate of the α wave, and 27 m.p.s. of the β wave. As will be seen later, the range of rates in the phrenic nerve probably is about the same as this proportionally.

At zero distance of conduction (*0 cm.*) these waves are all in phase and sum to form a high, brief potential wave such as is actually recorded at or close to the site of stimulation. The lags of the slower waves behind the fastest at the several distances of conduction, viz. 1, 2, 3–10 cm., and the effect these lags have upon the height and form of the composite action wave, are shown in the figure. As will be seen later on, the reduction in height, the prolongation of the rising and falling phases of the action wave and many other of the alterations presented by these diagrammed composite waves as the conducted distance increases, parallel very closely the alterations exhibited by the simple action potential wave as it moves along the nerve.

Summation of potentials in nerve trunks. It has been assumed above, and also in a previous publication (Erlanger, Gasser and Bishop, 1924), that if a certain number of axons in a nerve trunk are activated, the rest serving as a shunt across the leads of the apparatus, the recorded potential should be proportional to the number of fibers stimulated, if the potential of each axon is the same. It should then follow that the area under the curve of potential is proportional to the number of active axons, if the average potential of any group chosen is a constant, even if their action potential waves are not all in the same phase. The question involved in this assumption has now been put to the test of experiment.

If the sciatic nerve is mounted with lead-off electrodes toward the central end, and the dichotymous branches (tibial and peroneal) at the peripheral end mounted on separate stimulators, which, however, may be connected in parallel to the same coil, it is observed that the amplitude of the maximal action potential wave is almost exactly equal to the sum of the amplitudes of the two action waves led off separately, while the times to maximum of all three are equal, making the area under the curve of the two branches stimulated together equal to the sum of the areas when stimulated separately. On the other hand, when all the fibers are stimulated in the nerve trunk centrally, and the action waves led off from the branches, separately and together, the action current wave from each branch is equal to that from both combined.

This can only mean that one group of fibers, when unstimulated, shunts the action potentials of the other group in fairly close proportion to the number of fibers inactive, but when all are active and in the same phase, the reciprocal shunting effect of fibers is nil. In other words, although the recording apparatus acts as a potentiometer, the nerve trunk is so constructed that the effective potentials of its separate fibers act as if they were summed at the surface of the nerve trunk as a whole. The same reciprocal effect should obtain in fibers falling out of phase, in the nerve trunk.

THE RELATION OF AMPLITUDE TO DISTANCE OF CONDUCTION. *Theoretical considerations.* The diagrammatic representation of the action potential shows that during conduction the amplitude of the sum of the axon action waves falls off at two linear rates, figure 2B. The first and steeper gradient extends in this particular case over the first 6 cm. of conduction and connects at an angle with the second gradient. Constructions of various types show clearly, as does the one here produced, that the change to the less steep gradient begins where the slowest of the axon waves first begins to lag behind the crest of the summed waves. They show, furthermore, that, granting a uniform rate of conduction in each fiber, the distance of conduction at which the first linear gradient terminates depends upon the relative conduction rates of the constituent fibers and upon the duration of the axon action wave, and is independent of the relative heights of the several waves; the larger the ratio of the fastest to the slowest rate, and the briefer the duration of the axon waves the shorter is the distance of conduction before the discontinuity appears in the linear decline. The reason for this is that these are the two conditions that shorten the distance the slowest wave must travel in order to clear the crest of the summed waves. Under the conditions imposed, the amplitude-distance curve, up to the first discontinuity, must be rectilinear; but from then on it may be concave upward. This concavity will appear when there are *many* axon waves travelling at every rate between the two extremes, for then waves will be constantly clearing the crest of the composite wave.

Experimental data. The most favorable nerve for the determination of the relation of amplitude to distance of conduction is the phrenic, because of its length, its uniform diameter and the definiteness of the maximal response; also because its branches, if any arise from the part of the nerve used, are so small as to have no appreciable effect.

In figure 3 the amplitudes, *1*, recorded in one typical experiment each upon the phrenic nerve of the dog (dots) and the sciatic nerve of the bullfrog (crosses) are plotted against the conducted distances on the abscissae.

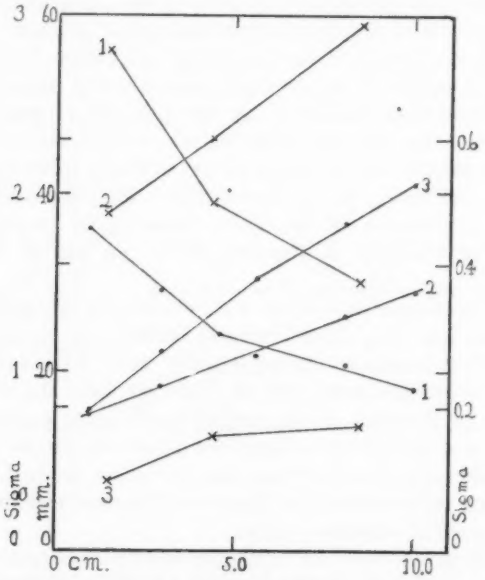

Fig. 3. Amplitude, *1*, duration *2*, and time to maximum, *3*, on the ordinates plotted against distance of conduction (abscissae). ● = phrenic of the dog (expt. 2, 14, '24, II); × = alpha wave in sciatic of the bullfrog (expt. 6, 19, 24). The time scale on the right side applies to phrenic *3*, only.

Though the number of points is insufficient to define any particular form of curve, it is obvious that their distribution permits one to draw through them two straight lines so as to obtain a curve resembling the amplitude-time relation curve derived in figure 2B. The point of contact between the two gradients if, indeed, one is justified in maintaining that the curves exhibit two gradients, is, of course, quite indefinite. But taking the curves for what they may be worth, one may say that the discontinuity occurs between 4.4 and 7 cm. and, in so far as this is concerned, that the conditions determining this discontinuity may be the same in the nerves as in the diagram.

DURATION OF THE ACTION WAVE IN RELATION TO THE DISTANCE OF CONDUCTION. The premised composition of the simple action potential of axon potential waves travelling at different but linear rates requires that the increase in the duration of the action wave with conduction be linear. Making due allowance for the difficulty of accurately measuring the length of the propagated action wave on account of the gradual approach of its termination to the base line, it is quite clear (see fig. 3) that the α action wave (crosses, *2*) and the phrenic action wave (dots, *2*) both increase in duration linearly with the distance of conduction. It might be added that this likewise is true of the entire compound action potential in the frog's sciatic (see fig. 2b, 3 and 4, Erlanger, Gasser and Bishop, 1924).

Granting the composite nature of the simple action potential wave, the linear increase in the duration of the wave during propagation may be taken to indicate that the rate of conduction in the slowest and the fastest fiber and presumably, therefore, in all of the fibers, is linear.[1] For if the rate in either the fastest or the slowest fiber were not linear, linearity of the increase in duration of the action wave would demand a parallel departure from linearity in the two fibers, an almost inconceivable condition.

The time to maximum in relation to the distance of conduction, according to the diagram (fig. 2C), should increase linearly and rapidly until again the slowest wave begins to lag behind the crest of the summed waves; then the rate of increase should fall off. The graphs of the relation of time to maximum to distance of conduction based upon experimental data show (see fig. 3, crosses *3*, for bullfrog and dots *3*, for phrenic) a rise that is rapid and presumably linear at first and then more gradual, the discontinuity occurring approximately at the same distance of conduction as in the case of the amplitude-distance curves.

ENERGY IN THE ACTION WAVE IN RELATION TO DISTANCE OF CONDUCTION. That the changes in the amplitude and in the form of the simple action wave during propagation are due to differences in the rates with which the individual axons conduct their action potentials is indicated by the well known fact that the energy as recorded in a galvanometer of the ballistic type does not decrease with conduction of the action wave. The equivalence of these two methods has been proved by registering in one and the same preparation the energy and the area of the action wave at different points along the nerve. Neither showed any change within the experimental error. In this experiment three stimulators were mounted upon a bullfrog's sciatic nerve in the unbranched peripheral region, at distances of 14.5, 44.0 and 85.0 mm. from the leading-off electrode, and all connected by switches to the same induction coil (fig. 4). The lead-off electrodes

[1] Evidence that this is true in the case of the fastest fibers is being presented separately.

were connected to a double-point double-throw switch, *a*, which led the action potential either to the amplifier and Braun tube or to a high sensitivity d'Arsonval galvanometer. The potential through the latter in series with the nerve, consisting of electrode imbalance and demarcation potentials, amounting together to about fifteen m.v., was balanced against a potentiometer, *b*, to a zero reading of the galvanometer scale. The action potential wave, produced by break shocks from each of the stimulators was recorded photographically (see fig. 5), and then, by a throw of the switch, the corresponding deflections of the galvanometer observed. The stimulus remained constant in strength and rate, and consisted of break shocks maximal for α, but below the threshold for β, preceded by a make shock that may have been above the α threshold, repeated thirty times per second. Six readings for each electrode were taken, with a new

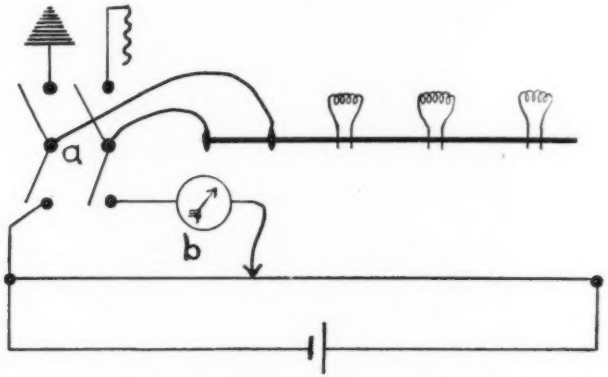

Fig. 4. Arrangement of nerve and apparatus for the records of figure 5 described in table 1. Stimulating electrodes connected through switches all to one coil.

zero between each two, to allow for drift of the galvanometer zero during the experiment due to imperfect potential balance. These readings, taken near the center of the scale, were proportional to the energy represented by the action potential, the make and break shock effects balancing out.

The photographic records of the action waves (fig. 5) were transferred from the semilogarithmic coördinates of the apparatus to linear coördinates, corrected to a rectilinear amplification characteristic, and measured with a planimeter. The results, collected in table 1, show, within the limit of error, that the area of the action wave as recorded by the oscillograph and its energy content as determined by the galvanometer remain constant during the propagation of the wave between points 14.5 and 85.0 mm. from the stimulating electrode. What discrepancy there is between different stimuli might well be assigned to different stimulating values of the current

at the electrodes. The correspondence between the areas and the deflections from the respective stimuli also is within the experimental error, and demonstrates the validity of the records of wave form from our apparatus.

Other possible causes of a decline in the amplitude of action potential, etc., with propagation, than unequal lags of potential waves in the con-

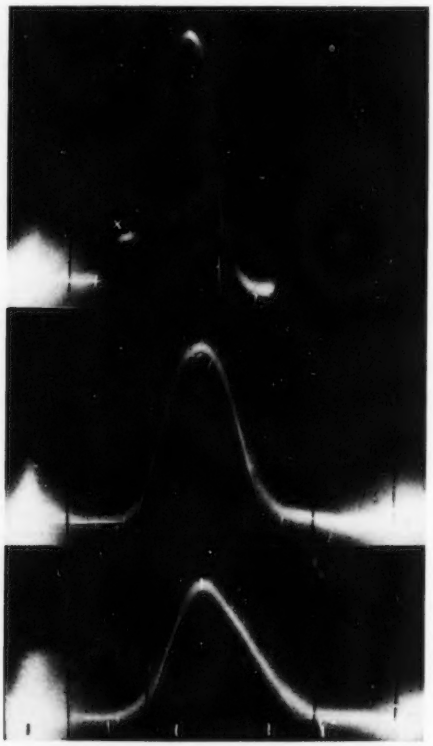

Fig. 5. Alpha action potential wave in the sciatic nerve of the bullfrog (6, 19, '24), 14.5 (top), 44.0 (middle) and 85.0 (lowest) mm. from the stimulus. x is the escape of the stimulus. The marks (black or white) on the base line of the lowest record indicate successive σ for all. $\times \frac{2}{3}$.

stituent fibers can be easily excluded. It cannot, for example, be laid to a physiological gradient, since the decrease in amplitude and increase in duration take place in either direction. Neither can it be assigned to conduction with a decrement, this explanation being definitely excluded by the failure of the energy in the action wave to diminish as it moves along the nerve.

THE BEGINNING AND THE END OF THE ABSOLUTELY REFRACTORY PHASE IN RELATION TO THE PROPAGATED ALPHA POTENTIAL WAVE. If the so-called simple action potential wave is composed of axon action waves travelling at different rates, then on the basis of their separation during propagation, certain predictable alterations should occur in the relation of the absolutely refractory phase to the phases of the action potential wave. In making these predictions two assumptions must be made, both of which, as will be seen, are quite defensible. The first is the immediate entrance into a refractory condition of all nerve fibers at the site of, and upon responding to, stimulation, in other words, with the start of the action potential in the individual fiber. Secondly, it is assumed that under normal conditions the absolutely refractory phase in any given fiber is constant in duration at all points along that fiber. These premises granted, it follows that despite the lengthening of the simple action potential wave as it moves along the nerve, the end of the unresponsive period will always fall a constant interval of time after the beginning of the action potential.

TABLE 1

STIMULUS	COND. DIST.	AREA OF REDRAWN CURVE	AVERAGE DEFLECTION	RATIO AREA TO DEFLECTION
	mm.		*mm.*	
1	14.5	0.222	63 ± 3	352
2	44.0	0.238	65 ± 2	366
3	85.0	0.231	61 ± 0.5	378

For, at any given point along the course of the nerve absolute refractoriness will cease immediately after the fastest conducting fiber ceases to be refractory and the refractory phase of the naturally stimulated fiber, we have premised, is the refractory phase of the artificially stimulated fiber. Furthermore, it must follow that, whereas at the site of stimulation the start of the composite action potential and of the absolutely refractory phase are coincident, at no other point along the course of the nerve will this be the case, because the individual fibers will become refractory as they become active; therefore, the nerve will fail to respond to a second stimulus applied to a point some distance from the point of application of the first only after *all* the fibers at the second point have entered into activity. Turning to the diagram (fig. 2), it is clear that the hypothetical nerve will become absolutely refractory to a second stimulus applied where it is the seat of an action potential wave that has travelled a distance of say 5 cm. from the site of the first stimulation, only after the start of the last component of the action wave has reached that point in the nerve, in this case 0.47σ after the beginning of the action wave. These predictions are verifiable by experiment.

For the determination of the end of the absolutely refractory phase in relation to either the unpropagated or the propagated action potential wave the nerve may be arranged on connections as shown in figure 6. To determine the end of the absolutely refractory phase at the site of stimulation the usual method is employed; the nerve is stimulated at A, first with a maximal induction shock from inductorium S_1, and then with another maximal shock from S_2, and the interval between these two shocks which just fails to cause a second response to appear at C is measured. To locate the end of the absolutely refractory phase with respect to the propagated action potential an action wave is started by stimulation of the nerve first at A and then at B while the action wave from A is passing B, adjusting the interval between these stimuli until, with the slightest further change in the time interval between them, a retrograde action wave appears or disappears at C'. Then, while leading through C, the position in the action wave from A of the escape of the second induction shock (the one applied at B), now made subminimal, is measured or recorded. The position of this escape in the action wave marks the end of the refrac-

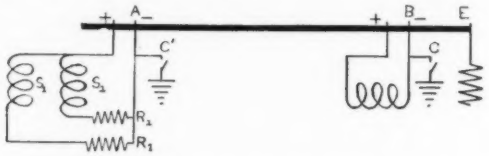

Fig. 6. Diagram of the method employed in determining the end of the refractory phase of the unpropagated and of the propagated action potential wave. Description in text.

tory phase with respect to the wave. This method of recording the second response at C' instead of at C, it might be added, has the advantage over the usual method, that by the time the action wave reaches C' the nerve has practically completely recovered from the relative refractoriness following in the wake of the first action wave (that from A), and the response consequently is unusually large.

Experiments of this kind show, in a word, that the absolutely refractory phase under our experimental conditions ends essentially the same interval of time after the beginning of the action wave at all points, including the one originally stimulated, along any given nerve. This confirms the conclusion of Bramwell and Lucas (1911) attained by subtracting the conduction time from the time separation of the two stimuli applied at different points along the nerve. The duration of the absolutely refractory phase in bullfrog's and green frog's nerve, that is, of the fibers quickest to recover, has ranged between 1.2 to 1.7σ (see fig. 9).

Upon comparing these figures with the duration of the action potential

wave it becomes obvious that the end of the absolutely refractory phase falls close to the end of the action potential wave as recorded at or close to the site of stimulation, but well up in the descending limb of the action wave that has proceeded any considerable distance from its site of origin. It should be added that this statement holds only in respect to the refractory phase of the fastest fiber to recover; for it has been shown in another place (Erlanger, Bishop and Gasser, 1926) that the slower (the γ) fibers of the bullfrog's sciatic may require as long as 4.46σ to recover their irritability. But, as has been said, it is only the quickest fibers to recover that determine the duration of the so-called absolutely refractory phase.

Location of the beginning of the absolutely refractory phase in the propagated alpha action wave. If, because of differences in the propagation rates of axon potential waves, the fibers at a point distant from the site of artificial stimulation become refractory in succession, beginning with the start of the action wave in each, stimulation of the nerve while and where it is in the early stages of a conducted action potential wave should have the effect of altering the form of that wave. The anticipated change in form can be made clear again by reference to figure 2. In the illustrative

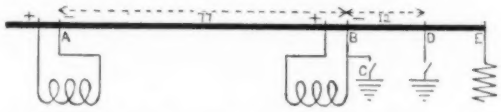

Fig. 7. Diagram showing the method of obtaining the records reproduced in figure 8. Description in text.

experiment to be cited below, the distance of conduction was 7.7 cm.; we will, therefore, consider here the conditions presented by the 8 cm. wave of figure 2. A stimulus applied under the conditions there portrayed, say 0.17σ after the beginning of the summed action wave (point indicated by the arrow), finds fibers *1* and *2* active. Since these fibers, therefore, are refractory, they do not respond to this stimulus and the part of the composite action wave determined by them, therefore, is not altered. But fibers *3*, *4* and *5*, which have not yet become active, are stimulated, and simultaneously, by this shock; consequently, the form of the summed action wave is changed by the advancement of these three waves to the point to which the stimulus is applied. The superimposed curve in the 8 cm. diagram presents the form of the action potential as altered by the summation of the axon waves thus displaced. The change in form consists of a steepening of the rising phase at the point of application of the stimulus, of an increase in amplitude, and of a shortening of the time to maximum and of the duration as compared with the unaltered summed wave.

The nerve (the sciatic of the bullfrog) for these experiments was arranged

on electrodes and leads as shown in figure 7. It was stimulated at *A* with an induction shock that was maximal for the alpha fibers but subminimal for beta; and, while observing simultaneously on the fluorescent screen of the Braun tube (a) the resulting action current as it passed lead *C*, and (b) the aerial escape into the amplifier of an induction shock from coil *B* (secondary circuit open so as not to stimulate the nerve), the time interval between stimulation at *A* and this escape from *B* was adjusted until the latter fell on the desired point of the passing action potential wave. Figure 8, *a*, pictures the position chosen in the present instance. Here the escape, *f*, appears 0.17σ after the beginning of the action wave, *a*, in the nerve under *B*. Then the action wave starting from *A* was recorded at *D*, 12 mm. further along the nerve. This action wave is shown as *b* in figure 8. A record was also made at *D* of the action wave started by a stimulus, again just maximal for alpha, but applied at *B*. This action wave, *c* (fig. 8), as the record shows, begins later than action wave *b*. This happens because it starts on its course 0.17σ after the start of action wave *b* had reached *B*. It, *c*, is shorter in duration and higher than action wave, *b*, because it has travelled a shorter distance. It is so much shorter than *b* that despite its later start it actually ends earlier than *b*. The difference in the ending time as exhibited by this figure may seem slight. It should be recalled, however, that by our method the time is recorded on a logarithmic scale, in which the length of the time units diminishes from left to right. When observations are being made which have as their purpose the determination of the shift of the end of the action wave, this phase of the deflection can be brought forward onto the quicker part of the line, where the same time difference may subtend from two to three times the distance shown in this case. To return from this digression, the time to maximum of the action wave *b*, it will be seen, is considerably longer than that of *c*. The crests of the two fall upon the same abscissa; this, however, is merely a matter of chance and is due to the fact that in this case the time chosen for the second stimulus happens to bring about this result. In other parts of the experiment, where the time interval between the beginning of the action wave and the application of the second stimulus was shorter, the crest of the second action wave actually fell earlier than that of the first.

Finally, while recording at *D*, the nerve was stimulated first at *A* and then at *B*, with stimuli both of the strength that produced the action wave in *c* and with the second stimulus so placed that it fell into the nerve again 0.17σ after the action wave coming from *A* had reached *B*. The record, *d*, (fig. 8) shows that the rising phase of the resulting action wave obviously is composed of a combination of *b* and *c*. It starts early and slowly as does *b* (the very beginning of the action current is covered by the escape, *n*, of the shock that produces *c*), but at the point corresponding with the start

of the action wave in *c* the record bends sharply upwards and then conforms in shape with the action wave shown in *c*, differing from it in one respect

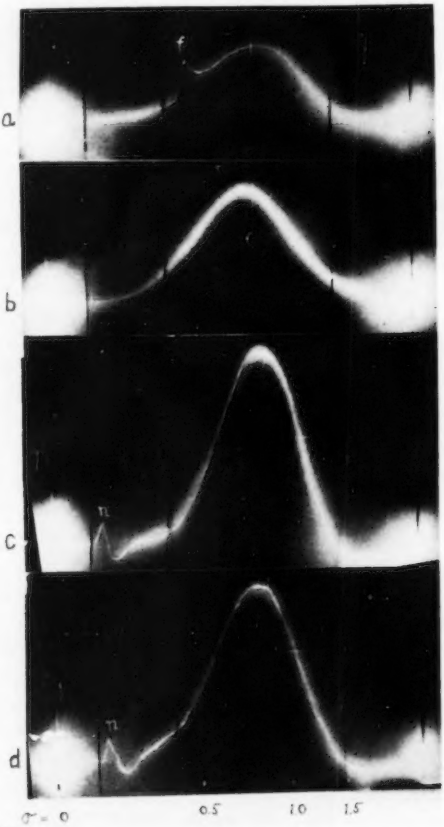

Fig. 8. Records from an experiment (6, 13, '24) demonstrating the absence of a refractory phase under the earlier stages of a conducted alpha action wave, bullfrog's sciatic. *a*, action wave from stimulus *A* (see fig. 7) recorded at *B*, together with the aerial escape of the induction shock (*f*) from *B*. *b*, action wave from stimulus *A*, recorded at *D*; *c*, action wave from stimulus *B* recorded at *D* (the latter was delivered so as to stimulate the nerve at the moment the action wave from *A* had developed to the point indicated by the escape, *f*, in *a*); *n* is the escape of the stimulus that started the action wave in *c*. The vertical rulings are on the face of the tube and indicate comparable time points excepting in *a*. $X = 8.5$ cm.; 1 mf.; 1,000 ω. Dots (black and white) on base line mark the time for all of the records. $\times \frac{2}{3}$.

only, namely, in that it is lower. The form of the action wave in *d* resembles very closely, it will be seen, that of the superimposed wave in figure 2,

A, 8 *cm*. Without doubt, therefore, the second stimulus applied at *B* has found most of the fibers of the nerve still irritable despite the fact that the nerve at that point is the seat of an action potential.

To determine in such an experiment the point, relative to the beginning of the propagated alpha wave, where all fibers finally become absolutely refractory, the second stimulus, the one applied at *B* in figure 7, is moved later and later in the action wave coming from the site of the first stimulus (from *A*, fig. 7) until the addition of the second stimulus (both stimuli being just maximal for alpha) causes no shift, be it forwards or backwards, in the position of the end of the action wave started by the first stimulus. This sign, it should be added, loses in clearness as the end point approaches, and therefore, is not especially precise.

The results of a typical determination of the beginning and of the end of the absolutely refractory phase in relation to a propagated alpha action wave in the bullfrog's sciatic may now be cited. The absolutely refractory phase at the site of stimulation ended at *E* (fig. 9a), 1.68σ after the first stimulus. It began at *B*, 0.58σ after the start of the action wave which had traveled 71 mm. It might be pointed out here that the beginning of the absolutely refractory phase of the 7 cm. action wave of figure 2 is 0.66σ later than the beginning of that action wave. The end, *e*, of the alpha action wave after conduction for a distance of 71 mm., determined by a calculation based on the action wave recorded after 88 mm. of conduction, the crest time, *D*, arrived at by an approximation, and the start, *O*, are joined by lines so that a triangle is formed having approximately the time relations of the action wave at a distance of 71 mm. from the site of stimulation, but of arbitrary amplitude. An axon potential wave is indicated similarly by the triangle *O A d*. The duration of this wave was derived from a record obtained by leading directly from the stimulating electrode, measuring from the start of the shock escape to the end of the potential wave and deducting 0.05σ, the assumed utilization period of the stimulus. The time to maximum of this wave was roughly estimated by a calculation based on data obtained from the action wave recorded at a distance of 17 mm. from the site of stimulation, the time to maximum in the record obtained at the site of stimulation being obscured by the escape. The same axon action wave (*O'A'd'*) is drawn into this figure again but with its end coinciding with the end, *e*, of the propagated action wave, *D*. If the simple action current is composed of axon action waves all of the same duration, the start of the triangle *O'A'd'* should coincide with the beginning, *B*, of the absolutely refractory phase of the propagated action wave. These two points, however, are separated by about 0.32σ. Later on some evidence will be given indicating that the more slowly moving axon potential waves of alpha may be slightly longer (up to 0.05σ) than the quicker waves. If this applied here the time subtended by *B* and *O'* would

be reduced to something like 0.27σ, a discrepancy which is within the error of the method of locating the beginning of the absolutely refractory phase.

Upon the assumption that either B or O' marks the beginning of the last of the axon potential waves entering into the composition of the action wave, D, the rate of conduction of the slowest wave can be calculated by the lag formula developed in a previous paper, the rate of the fastest wave in this case, directly determined, being 39.8 m.p.s. It is thus found that by the refractory phase method the slowest wave is traveling at the rate of 30 m.p.s., or, by the subtraction method, at the rate of 26.5 m.p.s.

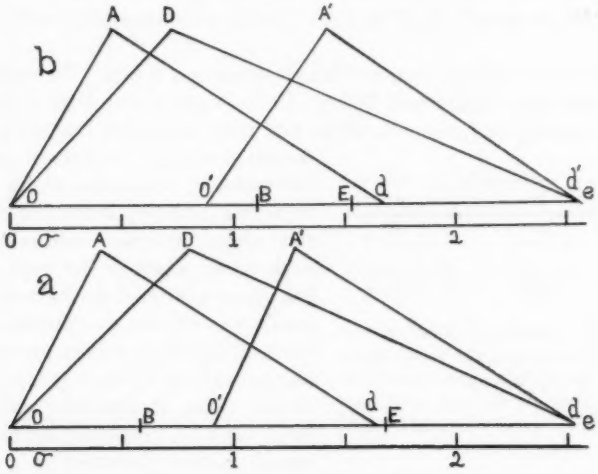

Fig. 9, a and b. Diagrammatic representation of the results of two experiments (6, 18, '24 and 6, 23, '24) on the relation of the end, E, and of the beginning, B, of the absolutely refractory phase of the propagated alpha action wave to the propagated wave and to the fastest and slowest moving of the axon action waves. The action waves are represented as triangles having the determined durations and times to maximum, but arbitrary amplitudes. ODe = alpha action wave propagated 7.7 cm. in a and 4.8 cm. in b; OAd and $O'A'd'$ = action waves as at the site of stimulation, placed with the start of one on the start of ODe and with the end of the other on the end of ODe. Time in *sigma* on base lines.

The results of a similar determination exhibiting, however, a disagreement in the other direction as regards the relation of the beginning of the absolutely refractory phase to the beginning of the last axon wave, are shown in figure 9b. Here the rates of the slowest axon wave, calculated on the basis of the refractory phase method and on the basis of the location of O', turn out to be 22 and 25 m.p.s., respectively.

It may be of interest to direct attention here to the shortness of the period

of absolute refractoriness under the action wave propagated these distances. This result is quite in keeping with the indications of figure 2A, which show that under the conditions there assumed, one might expect to find a complete absence of any refractoriness whatever under an alpha action wave that has traveled a distance of about 10 cm. This we have not confirmed by experiment because we have not examined a bullfrog's nerve 10 cm. in length with this in mind. It is, however, an easy matter to show experimentally that absolute refractoriness may be completely lacking under an action wave that has traveled less than 2 cm. when it includes the slowest (δ) axon waves, which may travel at a rate of less than 10 m.p.s. when the fastest have a velocity of 42 m.p.s. This is quite consistent with calculation.

ANALYSIS BY OPPOSING ACTION WAVE BY ACTION WAVE. In a preceding paper (Erlanger, Gasser and Bishop, 1924) it was shown that if an action wave consisting of maximum alpha and beta processes traveling in one direction along a nerve is made to encounter a maximum alpha process traveling in the opposite direction the two alpha processes mutually block each other, whereas the beta process continues unaltered on its way. This result was offered as conclusive proof that the alpha and beta processes are carried each in its own group of nerve fibers. Now, if, as all the evidence we have thus far presented indicates, the simple action potential is composed of action waves traveling in individual nerve fibers, fibers differing among themselves in respect to irritability also, the same general experimental method should likewise be applicable to the analysis of the simple action potential wave. There is, however, one respect in which the two cases are not quite alike; the analysis of the compound action wave yields perfectly definite and clearcut results because there may be at least the semblance of a margin between the just maximal strength of stimulation for alpha and the threshold strength of stimulation for beta, whereas no such margin exists between the irritabilities of the individual axons contributing to the potential of a simple action wave, such as alpha, in the frog's sciatic. Consequently, on account of the decrease in the stimulating value of the current from the point on the nerve in contact with the cathode to the point in contact with the anode, a so-called submaximal stimulus might stimulate fibers of low irritability at the favored point while failing to stimulate fibers of higher irritability that happened to occupy a protected position. It is obvious, however, that the weaker

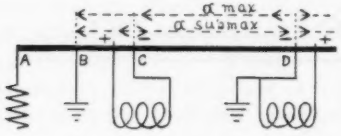

Fig. 10. Diagram indicating the method of analyzing the alpha process in the frog's sciatic nerve by passing a stronger through a weaker alpha process (expt. 11, 15, '24). Description in text.

the stimulus the smaller relatively would be the number of slowly conducting fibers whose threshold would be reached.

The anticipated as well as the actual results of an experiment (11, 15, '24)

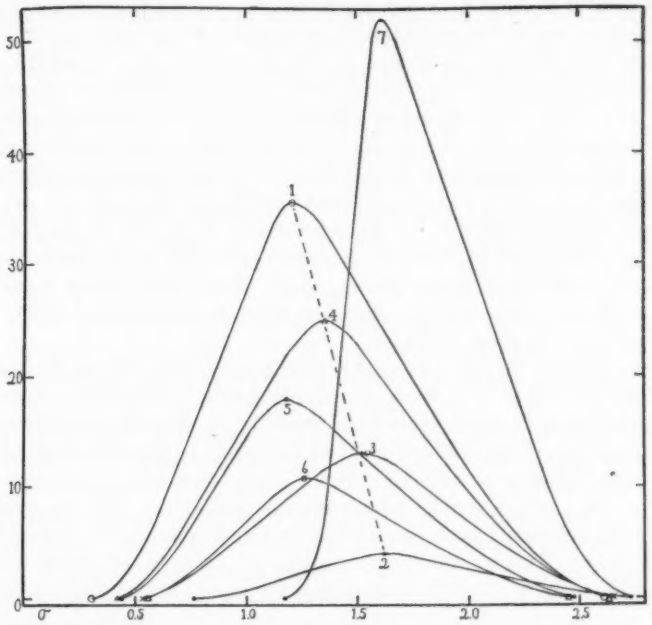

Fig. 11. Action waves, obtained by causing a descending alpha process to pass through an ascending alpha process, the nerve being arranged as shown in figure 10, drawn on linear coördinates in the same relative positions they (excepting 7) took on the fluorescent screen as a result of the changes in the conditions of stimulation.

1. (circles) = maximum alpha descending unopposed; 2. (dots) = maximum alpha descending, after passing through a just submaximal alpha ascending; 3. (crosses) = maximum alpha descending after passing through an alpha, ascending, of medium strength; 4. (triangles) = maximum alpha descending after passing through a threshold alpha ascending; 5. (dots) = submaximal alpha descending unopposed; 6. (squares) = same submaximal alpha descending after passing through somewhat weaker alpha ascending; 7. (dots) = maximum alpha descending after conduction 3 mm., restored, by certain corrections, to the form it probably had at the site of stimulation, and set with its termination on the termination of *2*.

Ordinates, amplitude in mm.; abscissae, time in sigma, zero being the position from which the fluorescent spot starts in each cycle.

based upon this principle will be presented side by side. The nerve (sciatic of *Rana pipiens*, very large specimen) is placed upon two pairs of stimulating electrodes, *C* and *D*, as indicated in figure 10, and, while leading

into the oscillograph through A and D, the nerve is stimulated at C and, in effect, a determination is made of the time required for the action wave to arrive at D. Then the apparatus is so adjusted that the nerve can be stimulated at D shortly before the action wave ascending from C reaches D. Having made this adjustment, arrangements are made to lead into the oscillograph through B and A instead of through D and A, and a contact print is then made of the action wave resulting from stimulation at D alone with a stimulus just maximum for alpha, the conducting distance being 43 mm. The positions of the start, crest and end of this action wave, determined by mensuration of the photographic record of the standing wave on the fluorescent screen of the oscillograph, are set down in a system of coördinates (fig. 11), and these points, for the sake of clearness, are here joined together by a curve so as to give to the figure the conventional form of an action wave. This gives action wave 1 (circles); it is made up of the sum of all of the alpha axon potential waves after conduction a distance of 43 mm.

Now, without changing any other of the conditions, the nerve is stimulated at C as well as at D, these stimuli being so timed that the action wave ascending from C meets the action wave descending from D close to D. The action waves are made to meet at this point so that before the arrival at B of the action wave from D there is time for the complete subsidence at B of the process resulting from stimulation at C. The interval thus obtained (calculated from conduction rate and distance) in this case was about 1.7σ. The stimuli applied through C were of three different strengths, namely, slightly submaximal for alpha, about midway between maximum and threshold for alpha, and just above the alpha threshold; the resulting action waves as recorded at B are plotted in figure 11, in the manner described above, as *2*, *3* and *4*, respectively.

The anticipated results of the experiment can be made clear by reference to figure 2. If it be assumed that the stimulating current reaches all of the fibers in equal strength, then, when the stimulus starting the ascending action wave is just below maximum for alpha, all of the descending axon action waves will be blocked excepting a small group traveling in the less irritable and more sluggish of the fibers. Such a combination might have the effect of eliminating, say, axon waves *1* to *4*, inclusive. The resulting action wave would then consist of wave *5* only. Since the distance of conduction in the illustrative experiment is over 4 cm. but less than 5 cm. the comparison can best be made with the 5 cm. wave of figure 2. At this distance of conduction the elimination of waves *1* to *4* would delay the start and the crest of the action wave 0.47 and 0.3σ respectively, lower its amplitude, but leave unchanged the location of its termination. The result actually obtained (fig. 11, *2*, dots) agrees with this, except (*a*) that the crest delay is longer by 0.1σ, and (*b*) that, apparently, the termination

is somewhat delayed (about 0.17σ). Discrepancy *a* would almost completely disappear if the diagram included a 6th wave traveling more slowly than *5*, and if the part of the action wave unblocked included axon waves *5* and *6*. If only the least irritable fiber had been left free to carry the descending action wave, a result which, naturally, is unattainable, the phases of the recorded action wave would have had the same duration as those of the action wave at the site of stimulation. With a view to ascertaining how close the agreement might be, such an action wave was obtained, not, however, at the site of stimulation, but 3 mm. removed from it. The record thus obtained, corrected for the alteration in form attributable to conduction, is drawn as *7* (dots) in figure 11, with its termination resting on the termination of the most completely blocked of the action waves, *2*. It will be noted that the crests of these two action waves fall approximately upon the same abscissa. The start of the blocked action wave, *2*, though, is definitely earlier (0.4σ) than that of *7*. Presumably these differences signify merely that whereas the unblocked action wave, *2*, consists mainly of the slowest axon waves, it probably includes in addition a few of the waves of medium velocities.

In connection with exception *b* (see above) it is necessary to bear in mind that owing to the gradualness of the start and of the end of an action wave the location of these points, and especially of the termination, is fraught with a considerable error. The error in locating the latter point certainly may be as large as ± 1 mm.; and since the ends of the action waves of this figure lie at a point in the time line where 1 mm. is equivalent to 0.08σ, it is probable that the range of the end points in figure 11, amounting to 0.17σ, is without especial significance.

To proceed, now in less detail, with the consideration of the experiment in hand, it is seen in figure 11 that as the strength of the stimulus at *C* decreases the amplitude of the residuum (*3* and *4*) of the same maximum alpha action wave descending from *D* increases, until, with no opposing stimulus from *C*, a maximal alpha process (*1*) is recorded. As the action wave descending from *D* increases in amplitude its crest moves forward, and almost linearly with the height, as indicated by the dotted line, the total shift amounting to about 0.4σ. In the diagram, figure 2, *5 cm.*, we have indicated by dots within circles the positions of the crests of the action waves composed of the last one, the last two, the last three, the last four, and all of the axon waves. It will be noted that these points, likewise, determine a straight line.

By means of a slight modification, the principle employed in the above experiment can be made to yield additional confirmatory evidence of the composite nature of the alpha wave. If the nerve be stimulated at *D* submaximally, instead of maximally, the more irritable fibers will respond, along, where the current lines are densest, with a few of the less irritable

ones. The crest of the submaximal action wave thus obtained (fig. 11, *5*) lies a bit earlier than the crest of the complete action wave, *1*. This is as it should be on the basis of the composite nature of the action current; the difference though (0.03σ) is almost within the limit of error. Due presumably to the lesser spread of the weaker stimulus the submaximal action wave, *5*, starts a bit later than the complete action wave, *1;* nevertheless it ends earlier, as it should if only the faster conducting fibers are responding. Next, a threshold action wave ascending from *C* is made to meet this descending submaximal action wave as in the preceding part of the experiment. The former should block mainly the more irritable of the fibers and consequently stop the fastest of the axon action waves descending from *D;* the action potential remaining should therefore be composed mainly of axon action waves of medium velocity. The record actually obtained (fig. 11, *6*), as compared with *5*, has a retracted start, and a slightly, though definitely, delayed crest, whereas the ends of the two almost exactly coincide: the result is quite consistent with the premised composite nature of the simple action wave.

The coincidences resulting from the comparison of the analytical data derived from the diagram upon the one hand, and from experiment upon the other can only mean that the assumptions upon which the diagram is based fairly accurately represent the conditions actually obtaining in the α group of fibers.

THE AXON POTENTIAL WAVE. *The time constants* of the axon potential wave can be determined directly by recording the action potential at the site of stimulation. This can be done by making the stimulating electrode the lead into the oscillograph. Under these circumstances, however, the records may be badly deformed by the overlapping escape of the stimulating shock; but by subtracting from the combined record the record of the escape obtained separately the form of the action potential wave can sometimes be derived (Erlanger, Gasser and Bishop, 1924). More recently we have succeeded in obtaining records at, or very close to the site of stimulation, in which the escape was so insignificant as to make subtraction unnecessary. This has been accomplished in at least two cases (see fig. 12, *a* and *b*) by employing a nerve of low threshold and without a connective tissue sheath (sensory root of the bullfrog and phrenic nerve of the dog with sheath dissected off), and by balancing the nerve circuit for resistance and capacity in a Wheatstone bridge (Bishop, Erlanger and Gasser, 1926). The escape of a shock just below threshold applied across the bridge is then insignificantly small (see fig. 12, *c*).

In confirmation of our previous determination, these records show that the action wave starts within 0.05σ of the start of the very short shocks delivered by the Porter coreless coil. The time to maximum and the duration of the action wave were, in the sciatic root, 0.42 and 1.50σ and

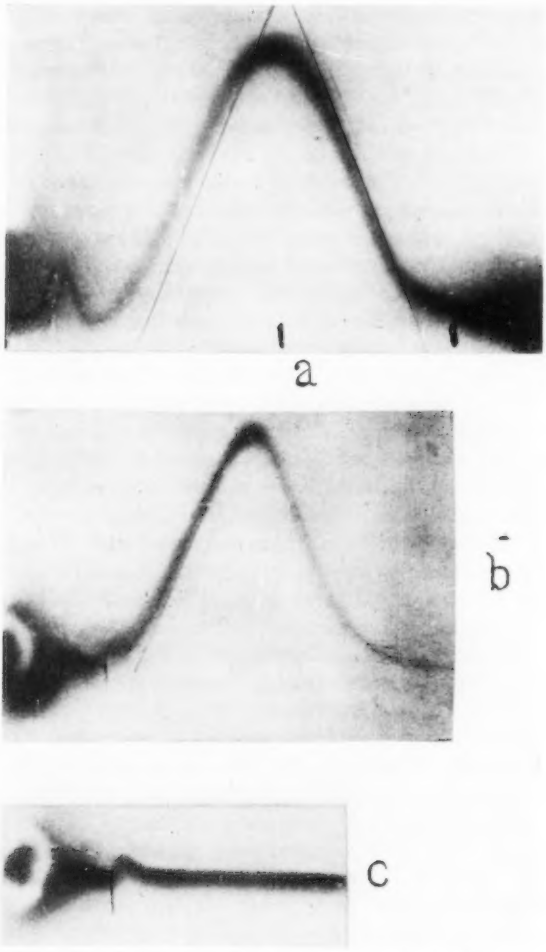

Fig. 12. The action potential at or close to the site of stimulation:

a. From a lumbar sensory root in the bullfrog (11, 22, '24) 3 mm. from the site of stimulation. The time elapsing between the start of the shock escape and the start of the action wave includes utilization period and conduction time. The time for conduction 3 mm. at the measured rate is 0.07σ. The utilization period therefore is 0.03σ plus an indeterminate time referable to the spread of the stimulus.

b. From a dog phrenic with sheath dissected off (2, 14, '24), leading directly from the stimulating electrode. The utilization period is not over 0.05σ.

c. The distortion produced by the shock employed in *b* reduced to just below the threshold value. Natural size.

in the phrenic nerve, 0.29 and 1.5σ. In another trial in the phrenic nerve, where, however, some deformation by the escape was evident, the time to maximum and duration were 0.18 and 0.71σ. The duration in the latter case was correctly given, but the crest of the action wave probably was displaced somewhat by the escape, and so as to make the time to maximum too short.

By this same method of recording the action wave at the site of stimulation it is possible to ascertain whether the axon action waves in fibers of low irritability are longer in duration than in the fibers of high irritability, for by leading from the stimulating electrode the complication is avoided of a shift in the locus of origin of the action wave as a result of current spread. To be sure, under these circumstances the reduction in the utilization period that accompanies an increase in the strength of the stimulating current will have the effect of producing an apparent reduction in the duration of the potential wave. But owing to the brevity of the utilization period in our experiments, not over 0.05σ, reduction in duration due to this factor will be negligible. Experiment shows that with the increase in the strength of stimulation the action wave as measured from the beginning of the induction shock apparently increases, though very slightly, in duration. The lengthening associated with an increase in the strength of the stimulus to one approximately maximal for alpha amounts only to 0.05σ. With further increase in the strength of the stimulus the duration of the action wave apparently increases about 0.12σ above the minimum. We say apparently, because at best it is difficult to determine exactly the end of the decline of the potential to zero; and the increase in the amplitude of the action wave that accompanies the increase in stimulation strength, by lengthening the ordinates of the curve, may have the effect of moving the recognizable end point back along the time line, thus giving the impression of an increase in duration by reducing the error in locating the end of the action wave.

The duration of the axon potential wave can be arrived at also, though, of course, very roughly, by an extrapolation based upon the assumption that the axons conduct at linear rates. It is given by the intersection of the time axis and the curve (a straight line, see fig. 3) determined by plotting the duration of the action wave against the distance of conduction. From the better sets of records the durations thus derived have ranged in the case of the dog's phrenic between 0.69 and 0.96σ, and in the case of the bullfrog's sciatic between 1.4 and 1.9σ.

It will be noted that the time to maximum and the duration of the axon action potential wave obtained by the above methods are somewhat longer than the values employed (bullfrog's sciatic, time to maximum 0.3, duration 0.9σ; dog's phrenic, 0.2 and 0.6σ, respectively) in constructing the diagram (fig. 2) of the simple action current. The values used were

adopted because, upon trial, it was found that they gave a better agreement between the various constructions and the results of experiment. With a view to ascertaining whether this discrepancy might be due to faulty recording we have carefully reinvestigated the extent to which our method might deform records. The results, presented in the appendix to this paper, prove that the action waves are correctly reproduced. Evidently, therefore, the discrepancy is due to the initial slow rise and the slow terminal fall of the axon potential wave, the very beginning and the very end of the axon potential waves contributing, consequently, relatively little to the total deflection.

THE RANGE OF CONDUCTION RATES OF THE AXON POTENTIAL WAVES. If the action potential wave of a given nerve is built up of units alike in duration and moving at different but uniform rates it should be possible to determine by calculation the rate of propagation of the slowest of the axon potential waves, given the velocity of the fastest fiber, which is the velocity of the potential wave as ordinarily determined, the duration of the whole action potential wave after conduction a given distance, and the duration of the axon action wave. The records obtained in the usual manner with the cathode ray oscillograph yield all of the necessary information except the duration of the axon action wave. The latter value can be obtained, as has been seen, either by recording the action wave where it arises at the site of stimulation or, indirectly, by a calculation based on the duration of the action wave obtained at two distances of conduction. By means of these methods, which are by no means free of error, it is found that the ratios of the rate of the fastest to that of the slowest component are about alike in the phrenic nerve of the dog and in the alpha wave of the frog's sciatic nerve. In two phrenic preparations the rates of the fastest and slowest axon waves have been found by this method to be 60 and 41 m.p.s. (2, 14, '24) and 73 and 51 m.p.s. (5, 16, '23), respectively. The ratios of the fastest to the slowest are 1.46 and 1.43. The limiting rates in the alpha wave in one bullfrog's sciatic were 40 and 24 m.p.s., the ratio being 1.67. It may be recalled here that the rate of the slowest α wave in the bullfrog's sciatic nerve as determined by the refractory phase method (22 to 30 m.p.s.) is in agreement with the results of this more direct method. It has been pointed out, furthermore, that if there is a stimulation interval between the slowest wave in alpha and the fastest in beta in the bullfrog's sciatic nerve it is very narrow. On this basis it seems justifiable to assume that the usual rates of the alpha and beta waves are the *outside* limiting rates of the alpha axon waves. These rates are about 42 and 27 m.p.s., giving a ratio of the fastest to the slowest of 1.55. Very rough determinations by the same method in two saphenous preparations of the dog give limiting rates in the α axons of 71.5 and 51.7, and 83.3 and 57.8 m.p.s. These yield ratios of 1.38 and 1.44 respectively. If there is

any significance attached to the similarity of these ratios in different nerves this remains to be determined.

THE LENGTH OF THE SIMPLE ACTION WAVE AS AFFECTED BY THE STRENGTH AND DURATION OF A STIMULATING CONSTANT CURRENT. Further evidence indicating that the fibers participating in the alpha process of the frog's sciatic nerve differ among themselves in a physiological sense is obtained by stimulating the nerve with a constant current of "infinite" duration. When the strength of such a current is varied between threshold and maximum for alpha and the resulting action waves are recorded at a distant lead it is found that the time required for the action wave to reach the lead-off electrode decreases very materially. At the same time the dura-

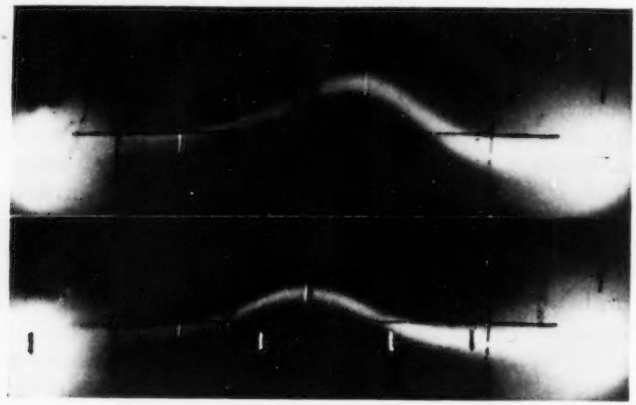

Fig. 13. Records, showing the shortening from behind of an alpha action wave in the bullfrog's sciatic as a result of shortening the duration of a threshold constant current of infinite duration, mounted so that vertical lines mark synchronous points. Upper, at infinite and, lower, at finite duration. Time in sigma indicated by black and white marks on base line of lower record. Natural size.

tion of the process also decreases. Both of these changes, we take it, are due to the earlier effectiveness of the stronger currents, which cause all of the fibers to respond earlier, the slower and less irritable fibers, however, to a greater degree than the quicker. That this is the correct explanation is indicated by the effect upon the action wave of shortening the duration of a constant current of any effective strength. Whereas strengthening of a current of infinite duration makes the action current both earlier and briefer (and also higher), shortening the duration of a current of given strength up to a certain limit merely shortens the action wave from behind (and lowers its amplitude) as shown in figure 13 (3, 21, '25). These experiments then obviously demonstrate that the fibers contributing to the alpha

process differ among themselves in respect to their utilization periods, the more slowly conducting fibers responding only to the currents of longer duration.

Discussion. It has been seen that all of our experimental findings are consistent with the view that the change in the configuration of the simple action potential wave and of the components of the compound action wave during propagation is due to differences in the rate of conduction of the action potential waves in individual nerve fibers. Certain of the results obtained by us, however, such, for example, as the decrease in amplitude of the action wave during conduction, might be taken to indicate that we are dealing here with the type of conduction known as decremental. Proof that this is not the case has been presented. But let us assume for the sake of argument that decremental conduction, the existence of which, even under abnormal conditions, has been called into question (Kato, 1924; Davis, Forbes, Brunswick and Hopkins, 1926) does obtain. Then the all-or-none law, the validity of which we have tacitly assumed, no longer holds. Summation of successive stimuli might, therefore, be possible and the change that is produced in the form of the conducted action wave by timing a stimulus so that it falls in the early phases of the action wave might then be accounted for as a summation of two stimuli in the individual fiber. In effect, this might be regarded as the substitution of an undecremented action wave for the portion of the decremented action wave which still has to pass the point where the second stimulus is applied.

There are, however, many difficulties in the way of the acceptance of this explanation of the change in the configuration of the action wave. One such objection is the fact that the action wave thus altered in form, is observed not only at the site of application of the second stimulus, but also at points beyond, to which the deformed wave is propagated. There is every reason for believing that the action potential is a unit process which is self-propagating; and it, consequently, seems most unlikely that anything but a uniform and simple process could be propagated by it. Just as stimuli of all kinds give rise to action waves of but one form, so even if it were possible to alter the form of a propagating action wave by means of a properly placed second stimulus, this complicated action wave likewise should be propagated as a wave of standard form only. Furthermore, if we were dealing here with summation, the quantity of current contained in the double action wave should exceed that in the single action wave. We find, however, that the weights of action waves *c* and *d* (fig. 8), when cut out of paper, are about alike (215 and 205 mgm. respectively), a result which is compatible only with the composite nature of the simple action potential wave. Finally, conduction with a decrement, with failure of the all-or-none law, would not account for the development of a delay in the

appearance of the absolutely refractory phase such as obtains in the case of the action wave after propagation. On the basis of conduction with a decrement either the nerve, it would seem, should not become refractory at all or should exhibit a refractory phase of the type observed in nerve at the site of stimulation, one beginning simultaneously with the action wave.

There is also to be considered here the possibility that a second stimulus applied to the nerve at a point where it is the seat of an action wave may polarize the nerve and thus lead to a distortion of the passing action wave. Such an electrotonic effect would be local, and an action wave deformed by it locally would as it moved out of the region of the nerve under the electrodes return to its normal form, which it does not do.

It is not impossible, however, to suggest for the propagated action wave a composition which might account for a delay in the onset of the refractory phase. If, as Lillie hypothecates (1914) (1922), the excited state propagates itself by means of a current flow through the adjacent inactive portions of the nerve, a flow determined by the potential difference between the active and inactive regions, it must follow that, of the potential wave as ordinarily recorded, the first (extrinsic) part occupies nerve which is as yet unstimulated, while the remainder (intrinsic part) develops in nerve that is responding and consequently is refractory. It should be a simple matter to demonstrate such an initial, unrefractory phase of the action potential wave if it has the duration, namely 1.0σ (length 3 cm., rate 30 m.p.s.), and the intensity ("sufficient to stimulate") premised by Lillie (1922). A short but adequate induction shock applied to the nerve where it is the seat of this extrinsic phase of a propagated potential wave should excite the nerve ahead of the aftercoming excited state. The excited state thus advanced should in turn develop in front of itself the extrinsic potential. Granting this, it would follow that if the action potential wave were recorded at some point further along the nerve, the wave started by the second stimulus mentioned above would appear at the lead ahead of where the wave started by the single (the first) stimulus would have appeared.

Figure 7 will serve to illustrate the arrangement of the electrodes and leads on the nerve used in experiments based on these considerations, if it is borne in mind that the distance between electrodes A and B is 12 instead of 77 mm. The sciatic nerve of the frog was stimulated first through the more distal (with respect to the leads into the oscillograph) pair of stimulating electrodes, A, with a shock just maximal for alpha, and, after an interval, with a shock through the proximal pair, B, so timed by trial as to reach the nerve while it was refractory as a result of the passage past B of the action wave coming from A. A second stimulus so placed alters neither the form nor (and it is this alone that is of significance here) the time of arrival of the action wave at the oscillograph lead, D.

Then the interval between the shocks from A and B was gradually reduced by causing shock B to come earlier, until the record gave evidence, in the form of a forward shift of the start of the action wave on the fluorescent screen, of the earlier arrival of the action wave at the lead D. Then the strength of the second induction shock was made subminimal and, by leading off of the cathode of the electrode pair, B (through C), the position of this induction shock in the action wave started by the stimulus delivered by the A pair of electrodes was determined.

In a typical experiment of this kind it was found, after reducing the interval between stimuli to the critical point mentioned above, that when the stimulus delivered by B was just above the threshold of alpha the shock appeared in the action wave from A and recorded at C only 0.02σ after its beginning. With a stronger B stimulus this interval became longer,—up to 0.1σ. This increase, however, is due to the spread of the stronger stimulus (Erlanger, Gasser and Bishop, 1924) and is of no present significance, only the results obtained when the second stimulus B is threshold being pertinent here. Indeed, it is more than likely that even so-called threshold stimuli spread somewhat. It is worth mentioning that if, for instance, the spread extended only 0.5 mm., which in all probability it does, the new action wave would start 0.015σ ahead of the applied electrodes. On the other hand, currents act for a finite time before they stimulate. In the case of the very brief induction shocks we employ, this so-called utilization period, as has been seen, is not over 0.05σ. The utilization period here might be shorter even than usual because the two stimuli, propagated and applied, possibly sum. We will, however, accept for present purposes 0.05σ as the utilization period. To ascertain the duration of the part of the potential wave ahead of the excited region we may then proceed as follows: 0.02σ (determined time difference between the start of the conducted action wave and the start of the second shock) $+ 0.05\sigma$ (the utilization period of the second shock) $-$ a factor of unknown magnitude resulting from spread of the second stimulus $= 0.07\sigma$. This result proves that if any part of the action potential wave is made up of eddy currents spreading into nerve yet unstimulated by the propagated disturbance, the length of such extrinsic currents probably does not exceed 2.9 mm. (time 0.07σ, propagation rate 42 m.p.s.). It is obvious, therefore, that spread of the action potential along nerve is a negligible contributory factor, if it contributes at all, to the initial unrefractory period of the propagated action potential wave. In view of the minuteness of the one determined value, of the dubiousness of the assumed value, and of the omission of spread of stimulating current as a factor, we do not care to claim that an accurate determination has been made of the extent of spread of the action potential, if, indeed, there be any.

Brief reference may be made here to the significance of these results

relative to the Lillie hypothesis of nerve transmission. The value arrived at above is too large; but if for the sake of discussion we assume that there is an extrinsic effect lasting 0.1σ, the excited state, which has a time to maximum (as measured at the site of stimulation) of 0.3–0.4σ, would add to its start an extrinsic effect 0.1σ in duration, as recorded after propagation. The maximum extrinsic effect would then constitute less than $\frac{1}{4}$ to $\frac{1}{5}$ of the rising phase of the hypothetical axon action wave as propagated. Its potential would depend upon the form of this wave, which is unknown, but from comparison with the unpropagated axon wave we may assume that it would amount to less than $\frac{1}{5}$ of the total potential. Therefore, if, as Lillie maintains, transmission of the excited state in nerve is by means of the direct influence of the current flowing from the excited region, a current having not more than one-fifth the intensity of the action potential and reaching its maximum in less, probably very much less, than 0.1σ must suffice to stimulate nerve.

The results of the entire series of experiments demonstrate conclusively that the so-called simple action current in fact is not simple, but is a composite of axon potential waves traveling at slightly different rates. Not only does each fiber conduct at its own rate, but in addition each apparently differs from every other in respect to its utilization period, its irritability and possibly in other aspects also. These, it may be recalled, are also the differences distinguishing the groups of fibers concerned with the production of the waves of the compound action current, which we have designated alpha, beta, gamma and delta. Now it has been seen that the slowest fibers of the alpha group probably travel at about the same rate as the fastest of the beta fibers. This leads one to infer that if there is a clear gap between the alpha and the beta fibers in respect to irritability and in respect to the rate with which they conduct their action waves it must be very narrow. We have recently looked very carefully into this question. If one plots the areas of the action waves, determined by planimeter measurements of the action wave records from the frog's sciatic nerve drawn on linear, rectangular coördinates, against the strengths of the stimuli, in terms of primary amperage, one usually finds that the transition from alpha to beta is indicated merely by a temporary slowing of the rate of rise of the curve. In one instance only have we succeeded in finding in such a curve a horizontal section between the maximal stimulus for alpha and the threshold stimulus for beta. It extended over about 10 per cent of the current increase between the threshold and maximum stimuli for alpha.

The further rise of these curves accompanying the increase in the strength of stimulation beyond that necessary to evoke alpha and beta is relatively slight, so slight that by this method it is a very difficult matter to settle the question of the presence or absence of gaps between the beta and the gamma, and the gamma and the delta fibers; but, generally speak-

ing, it would seem that the situation here is about as it is in the case of alpha and beta: either the gradient of the curve merely becomes temporarily less steep or, at most, horizontal for a brief interval.

SUMMARY

Evidence is presented indicating that the simple action potential wave, including in this term the constituent waves of the compound action potential, itself is composite and is formed by action waves (*axon action waves*) travelling at slightly different rates in the individual component fibers. This evidence is as follows:

a. Though the action wave during propagation diminishes in height, its duration increases correspondingly, so that its area remains constant.

b. In confirmation of this it is found that the amount of current, as determined by a slow-moving galvanometer, remains constant despite the change in the form of the action wave during propagation.

c. Despite the change in the form of the simple action wave during propagation, the time intervening between the beginning of the wave and the end of the absolutely refractory phase remains constant.

d. The beginning of the absolutely refractory phase is coincident with the beginning of the action potential only at the site of the initiating stimulus. In the propagated action potential wave the beginning of the absolutely refractory phase is later than the start of the action potential by an interval that can be accounted for on the basis of the construction of the action wave out of a large number of more or less uniform waves having about the duration of the action wave at the site of stimulation and travelling at more or less uniformly distributed rates, the beginning of the last of these axon waves coinciding with the beginning of the absolutely refractory phase.

e. By causing two action waves, each consisting of the alpha process only, but started by stimuli of two different strengths up to just maximal, to travel toward each other and meet, a type of blocking is obtained that can be accounted for only if the fibers participating in the alpha process differ among themselves in respect to irritability; and the configuration of the resulting action waves is such as to indicate that the less irritable the fiber the slower is the rate with which it conducts its action wave.

f. Evidence has been obtained indicating that the fibers contributing to the simple action wave differ among themselves in respect to their utilization periods, also.

Direct and indirect estimations of the time to maximum and of the duration of the axon potential wave in the bullfrog's sciatic yield values at room temperature of about $0.4+$ and $1.4+\sigma$, and in the phrenic nerve of the dog at body temperature of $0.29\pm$ and $0.7+\sigma$, respectively.

The time constants of all of the axon waves in a given nerve are approxi-

mately alike, though it is possible that the wave in the least irritable and slowest fiber in the sciatic of the bullfrog is a trifle longer in duration (but not over 0.12σ) than the wave in the most irritable fiber.

The range of propagation rates of the axon potential waves in the alpha process is almost, if not quite, continuous with the range of the rates in the beta wave; and, by inference, the same relation obtains between beta and gamma, and gamma and delta, also.

Practically all of the experimental data derived from the study of the alpha wave in the sciatic nerve of the bullfrog correspond with the conclusions reached by inference through the analysis of a diagram constructed of waves having a time to maximum of 0.3 and a duration of 0.9σ, and traveling at uniformly distributed rates ranging between 42 and 30 or 27 m.p.s.

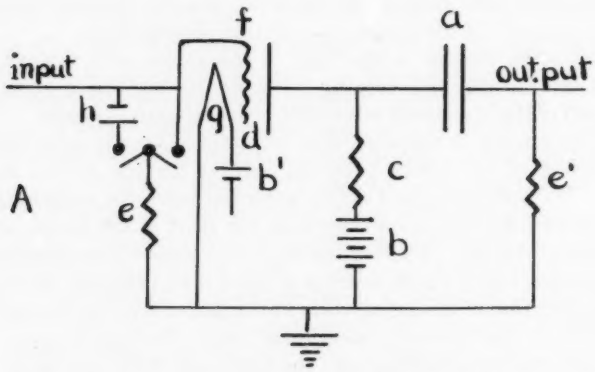

Fig. 14. Diagram of third amplifier panel, to indicate locus and nature of distortion in the amplifier with large input values, and with abrupt changes of input potential. For explanation and method of correction, see text.

If there is any spread whatever of potential ahead of the refractory region under the propagated axon action wave it does not exceed 0.07σ in duration or 2.9 mm. in length, when the action potential wave is travelling at the rate of 42 m.p.s.

APPENDIX. *Distortion.* The question naturally arises, how precisely the action wave as registered by the apparatus traces the potential curve of the nerve. The only distortion that need concern us is a time lag, since potential amplitudes are taken care of by calibration. The Braun tube oscillograph has no appreciable inertia, as may be demonstrated by applying to its terminals the make or break of a galvanic current.

In the amplifier, however, occurs a time lag due to capacity, to leak resistances and to the accumulating grid charges (fig. 14). The condenser, *a*, maintains a poten-

tial which depends on the flow of current from the battery, b, through a fixed resistance, c, and a variable tube resistance, d; this condenser is drained by the very small leak, e', from its other terminal. When the tube resistance, d, changes, the potential of a would come to a new equilibrium instantaneously except for the fact that first, the connecting output condenser, a, and its leak, e', act as a capacity and resistance,

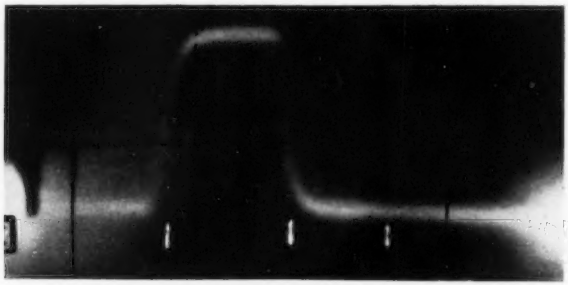

Fig. 15. Distortion of a rectangular input potential (instantaneous change of constant current) by the amplifier. Time marks are 0.25σ apart. Photographed black lines are deflection ecördinates of tube. For further explanation, see text. Natural size.

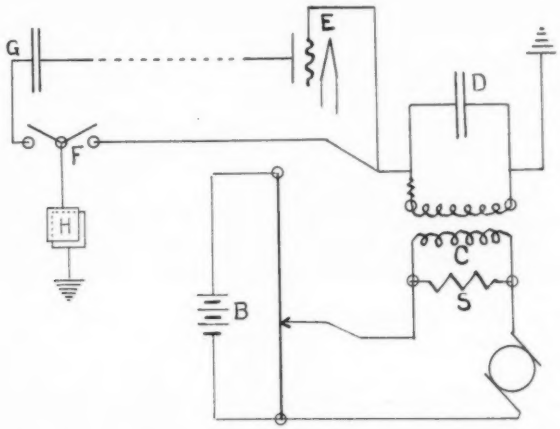

Fig. 16. Diagram of connections to determine distortion by the amplifier of a sine wave of the dimensions of an action potential wave. For explanation, see text.

connected in series, parallel to the plate circuit ($b\ g\ d$). The resistance, e', is kept large in order to minimize this effect. Secondly, the grid, f, tends to acquire the potential of the space charge in the tube due to the circuit from the plate, d, to the filament, g. When the input potential of f is changed, this space potential also changes. Since the plate circuit of the preceding panel and coupling condenser form a parallel circuit to the leak, e, the attainment of equilibrium of f to the changed

input conditions is retarded. The leak, e, must be low in resistance relative to the preceding plate circuit resistance to minimize this effect. The value of e is therefore in practise an empirical compromise accommodating the tube to these two conditions. The distortion appears in the record of the make or break of a galvanic current, as a deviation from a vertical line. The record starts without lag, but approaches its maximum logarithmically. Such a curve is reproduced in figure 15. The curve attains 90 per cent of its maximum in 0.000,03 second and 97 per cent in 0.000,07 second.

While an instantaneous or "rectangular" current is distorted materially, a curve that rises more slowly is distorted less, and one as slow as an action wave, almost not at all. To determine the lag of the crest of a curve, such as an action wave, a decremental sine wave was employed. The primary of a coreless Porter coil, C

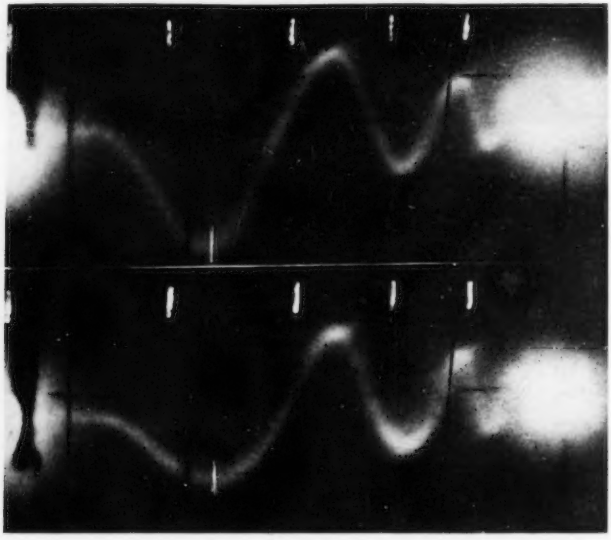

Fig. 17. Records of decremental sine wave, (lower) through amplifier, and (upper) on oscillograph direct. Time marks are 0.5σ apart. For description, see text. Natural size.

(fig. 16), was connected in series with the commutator, and its terminals short-circuited with a Helmholz side wire, s, of 1 ohm resistance, to avoid arcing and consequent varying resistance in the commutator. Current was led to this from a slide wire and batteries, B, such that the potential could be varied from a few thousandths of a volt to ten volts. The secondary of 300 ohms resistance was connected in series with a 0.025 m.f. condenser, D, and in parallel with the amplifier input, E, and the ground. The secondary coil was drawn away from the primary to avoid capacitative coupling, and turned at an angle of about 80°. A lead was made from the grid to one pole of a double throw switch, F; the opposite pole of the switch was led to the amplifier output, G, and the middle pole to the Braun tube input, H. Since the amplification of the apparatus is at present about 6,000, potentials in the

ratio of 1 to 6000 were applied to the primary, and the resultant oscillations recorded, respectively, through the amplifier, and on the tube directly by a throw of the switch, *F*, with no appreciable alteration in the oscillating circuit.

The result was a series of damped oscillations in each case (fig. 17), of about the same amplitude with (lower record) or without (upper record) the amplifier. One complete oscillation corresponds to an action wave of average duration (time to maximum 0.5σ, descending phase 1.0σ, total duration 1.5σ). Except for amplitude distortion on one side of the base line (taken care of by characteristic corrections), the two curves are almost superimposable, and the lag of the crests of the oscillations through the amplifier is not more than 0.02σ. This may be taken as the apparatus error in measuring crest times. It is a systematic one, not affecting relative values in a series of records.

It may be emphasized that the time of the *start* of any process is not distorted at all, within the limits to which we can measure. The lag of any other event of the action wave is well within the experimental error of our measurements.

It has been stated that amplitude distortion is taken care of by calibration. This is true within reasonable limits. Action potentials (or other input potentials) of unusual amplitude or duration (that is showing a large "area under the curve") tend to cause an increase of potential on the grid of the third panel, as described above, which acts as a permanently shifted grid bias in this panel, and is exhibited in the record as a shift in the base line, accompanied by a change in the amplification factor. This has been corrected approximately within the limits of our input values by reducing the resistance of the grid leak, *e* (fig. 14a), of the third panel from a former value of 500,000 ohms to 100,000 ohms, to prevent piling up of grid current, and by placing a -1.5 volt grid bias, *h*, between grid and ground, to reduce the value of the grid current. This error is thereby reduced to a negligible factor, though the total amplification is materially cut down by the adjustment. When large amplification is desired with small input values the instrument may be used without these changes, making use of the appropriate calibration curves.

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THE ACTION POTENTIAL WAVES TRANSMITTED BETWEEN THE SCIATIC NERVE AND ITS SPINAL ROOTS

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In preceding papers from these laboratories it has been shown that the sciatic nerve is composed of some four groups of fibers distinguished more or less sharply, one from the other, by differences in irritability and in conductivity (Erlanger, Gasser and Bishop, 1924). The fact of this grouping, particularly on account of its constancy, very naturally suggests the thought that there may be a corresponding segregation of fibers on a functional or developmental basis. Accepting this idea as a working hypothesis, the first and most obvious step to suggest itself was to obtain for comparison the action potential waves in the ventral and dorsal roots. This paper records the results obtained in experiments designed with a view to tracing the action waves from the sciatic nerve into the dorsal and ventral roots of some one of its pairs, and from each of the roots of a pair into the sciatic nerve.

METHODS. The general methods used in these experiments have been described in previous publications (Gasser and Erlanger, 1922), (Erlanger, Bishop and Gasser, in press); the amplified action potentials were recorded by means of the cathode ray oscillograph. The preparations employed consisted of the sciatic nerve with one pair of spinal roots attached. They were obtained for the most part from the bullfrog, a few from very large specimens of the green frog, and from the cat.

The bullfrog's sciatic, on account of its large size and its viability, has best served our purposes; therefore that preparation alone will be described in detail. When very long preparations were needed the roots in the brain-pithed frog were first laid bare and, by means of electrical stimulation of the motor roots, the pair was identified whose fibers extended farthest down the leg. This pair, usually the IXth, was then carefully removed together with the attached sciatic nerve and mounted in our moist chamber on stimulators and leads arranged for the most part as indicated in figure 1.[1] When maximum length of conduction was not essential the largest pair of roots, usually the VIIIth, was removed, together with the

[1] The first dissections were made by Dr. Moritomi Kubo of Keio University.

trunk formed by their junction, down to the point where it is joined by the trunks formed by the other two pairs of roots contributing to the sciatic nerve (see fig. 1). The only fibers not extending the entire length of the latter preparation are those leaving in the outflow through the dorsal branch, *d*, arising close to the exit of the nerve from the intervertebral foramen and in a minute branch, *c*, further down, communicating with the sympathetic chain. In the longer preparation, the trunk formed by the pair of roots removed is enlarged by the union with it of the remaining constituents of the sciatic nerve. But there is very little further outflow of fibers, especially in the case of the IXth pair, down to the origin of the first large muscle branch, *m*, from the sciatic. Afferent stimulation even as far along the nerve as this point, therefore, reaches a great majority of the

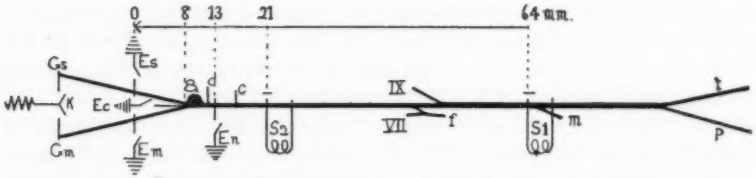

Fig. 1. Diagram of the sciatic nerve of the bullfrog with one of its pairs of roots (VIII) arranged on the leads and stimulators most commonly employed in experiments tracing the action wave from nerve trunk into roots. The distances here given are specifically those obtaining in experiment 5, 14, '25. *g* = ganglion; *d* = dorsal branch; *c* = branch communicating with sympathetic chain; *VII* = 7th spinal nerve; *IX* = 9th spinal nerve; *f* = femoral nerve; *m* = first large muscular branch; *t* = tibial nerve; *p* = peroneal nerve. S_1 and S_2 = stimulators; E_n, E_c, E_s and E_m = ground leads on nerve trunk, at crotch of roots, on sensory root and on motor root, respectively; only one of these leads is in contact with the nerve at a time. G_s and G_m = grid leads from ends of sensory and motor roots; through the switch, *k*, these can be connected with the recording mechanism either separately or together.

fibers of the root under observation and consequently produces practically maximum action waves, especially when the root pair is the IXth. On the other hand, the action wave started by stimulation of either of the roots when led from any point distal to the sciatic plexus is very much reduced in size through shunting by the many fibers that come from other than the stimulated root.

The motor and sensory roots, which usually were 15 to 20 mm. long, were tied, by ligatures fastened to their cut ends, each to a nonpolarizable electrode (G_m and G_s, fig. 1) of the type previously described (Erlanger, Bishop and Gasser, in press). These nonpolarizable electrodes, by means of the switch, *k*, could be put, either separately or together, into connection with the grid of the amplifier circuit. So as to have the full length of the root available for experimentation, special killing of the cut end occasion-

ally was omitted. In most of those instances the action waves, nevertheless, were monophasic. The grounded lead, E, could be connected with any desired point on the nerve without opening the moist chamber. The relative positions this electrode most frequently was made to occupy on the nerve are indicated in the diagram. Thus E_n is a lead from the *nerve* a few millimeters distal to the spinal ganglion, G, and consequently to the crotch formed by the junction of the roots. The leads E_m and E_s on the *motor* and *sensory* roots, respectively, were a few millimeters, and equally, central to the root junction. The lead E_c is from the angle of the root *crotch*, which, in our preparations usually was just central to the spinal ganglion. Since the action potential is recorded as its *leaves* the lead (proof to be presented in another place), and since the lead is about 1 mm. wide, the crotch lead actually is about 1 mm. central to the crotch. When this arrangement was used the cathodes of the stimulating electrodes were always on the side nearest the leads. S_2 stimulates practically all of the fibers going into the roots, though even as far down as S_1 the nerve still contains practically all of the fibers entering the ninth pair of roots. By means of switches outside the moist chamber these stimulators could be connected either with the same or with different coreless Porter inductoria.

The motor component of the action potential started by either of these stimulators could be recorded through the combination $E_m - G_m$, the sensory component through $E_s - G_s$. It was at first believed that the two components, as they passed the point E_n in the nerve, might also be recorded separately by employing the combination $E_n - G_m$ or $E_n - G_s$, respectively; and that by the combination $E_n - G_s G_m$ a picture might be obtained of the combined action potentials at E_n, in the nerve trunk itself. It was found, however, that while the combination $E_n - G_m$, for example, does give the motor root component it also records a small diphasic sensory action wave, the motor root, evidently, acting as an extension from the grid lead putting the intact side of the sensory fibers at the crotch in connection with G_m.

The records led from E_n were complicated in still another, and a considerably more subtle way. Not infrequently when leading at E_n, under conditions which should have given an action wave of the usual simple form, the record turned out to be deformed by a hump. It was finally discovered that this deformation is due to what amounts to a double ground lead from the nerve. The nerve trunk is covered by a relatively thick connective tissue sheath, presumably of high resistance. It is even possible, indeed, that occasionally in removing the retroperitoneal portions of the nerve, some of the very thin peritoneal covering inadvertently is left on the nerve and by intervening between the nerve and the lead still further adds to this resistance. The roots on the other hand have at most a very thin sheath; their fibers, therefore, are in effect in connection with

E_n through the layer of salt solution that moistens the nerve. Under these circumstances the ground lead in effect is double, and the two grounded points are in connection with each other externally through this salt solution resistance; the one grid electrode, though, serves as the second lead for both. The proof that the deformation is due to an action potential twice recorded, in a word, consisted in showing that moving the lead E_n away, for instance, from the position of the root junction caused the first of the two deflections to move into an earlier position, corresponding with the shortening of the distance between the lead and the stimulator, whereas the position of the second deflection remained unaltered.[2] This difficulty has stood in the way of an exact comparison of the combined motor and sensory action waves in the nerve with the waves as registered separately in the roots.

RESULTS. Briefly the results we have obtained relative to the distribution of the fiber groups between the roots were exactly those we had anticipated. The action potential in the motor root obtained in this way proves to be simple, while the waves characteristic of the conducted sciatic action potential run into the sensory root (see fig. 2). In addition, it has come to light that the sensory action wave suffers a slight delay in passing through the spinal ganglion.

Before presenting the evidence upon which the above findings are based it should be pointed out that not all of our experiments were completely successful. In every frog experiment active preparations were obtained, but some of these deteriorated rather rapidly, as evidenced by gradual failure of the roots to conduct. Apparently the roots are much more susceptible to untoward conditions than is the nerve trunk. This we found to be particularly true in the cat. Of the two preparations obtained from this animal under conditions which gave every expectation of success, one had an active nerve trunk, but roots which failed to conduct; the other gave satisfactory records through the crotch lead but nothing from any other of the root leads.

Table 1 contains all of the relevant data of the experiments in which a comparison has been made of the action waves running into or from the two roots. In one of the experiments (5, 14, '25) the roots remained irritable, and the results constant, over a period of $1\frac{1}{2}$ hours. The numerous records (26 in all) obtained during the course of this experiment demonstrate all of the essential facts of the entire series. Figure 1, illustrating the general arrangement of the connections on the nerve in these experiments, gives specifically the arrangement and distances of conduction obtaining in this particular experiment.

[2] A discussion, from a more general standpoint, of the deformation of action potential waves, due to such circumstances as these, is being presented separately.

TABLE 1

EXPERIMENT	ANIMAL	ROOT PAIR	DISTANCE OF CONDUCTION		LAG σ	TIME TO MAXIMUM		REMARKS
			Total	In root		Sensory σ	Motor σ	
			mm.	*mm.*				
11/ 8/24	Bullfrog	IX			Yes			No records
11/22/24-I	Bullfrog	VIII	118.5	5(?)	0.18	0.65	0.89	Stimuli maximal for α
11/22/24-II	Bullfrog	VIII(?)	110.0	(?)	0.15	0.89	0.73	Stimuli strength ?
5/14/25	Bullfrog	VIII	13.5	0	0.13	0.49	0.42	
			21.5	8	0.16(?)	0.83	0.51	
			56.0	0	0.13	0.59	0.49	
			64.0	8	0.11(?)	1.08	0.58	Stimuli maximal plus for β
			17.5	4	0.17	0.66	0.46	
			60.0	4	0.12	0.76	0.55	
			17.5	4	0.15	0.60	0.41	
			13.5	0	0.10	0.50	0.43	
6/ 5/25	Bullfrog	(?)	50.0	2	0.02			Stimuli strength ?
			49.0	0	0.09			
6/17/25	Bullfrog	IX	36.0	6	0.14	0.37	0.44	Conduction descending
			12.0	6	0.18	0.29	0.35	T = 27°C.
			33.0	3	0.16	0.39	0.45	V = 52 m.p.s.
10/27/25	Bullfrog	IX	48.0	(?)	0.18	0.75	0.76	Stimuli maximal for α
11/19/24	Green frog	(?)	70.0	2	0.14	1.32	0.84	Stimuli maximal for α
1/ 7/25	Green frog	(?)	61.0	(?)	0.08	0.82	0.51	Stimuli maximal for α
			17.0	(?)	0.04	0.48	0.38	Ganglion *in situ*
1/22/25	Green frog	VIII(?)	26.0	(?)	0.10	0.63	0.49	Ganglion *in situ*
1/24/25	Green frog	(?)	69.0	2	0.06(?)			Stimuli strength ?
			7.0	2	0.04	0.74	0.42	
2/ 5/25	Cat	D VII	25.0	0	0.08	0.38	0.34	Stimuli strength ? T = 35°C.

The action potential from the sciatic nerve is simple in the ventral, and compound in the dorsal root. When sufficiently strong stimuli are applied to the nerve at S_1, that is, at a point distant from the roots, and leads are

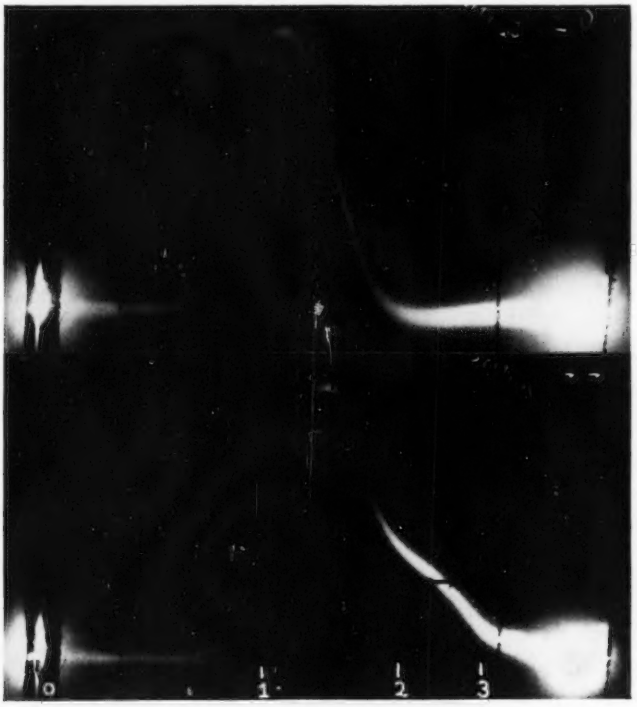

Fig. 2. Records from experiment 5, 14, '25 showing the action potential in the ventral root (upper) and in the dorsal root (VIII) (lower) of a bullfrog's sciatic preparation. The nerve was stimulated 56 mm. from the root crotch (see figure 1) and the leads from the roots were both exactly 4 mm. central to the crotch. The stimulation strength was the same, and maximal, for both records. The start of the action wave in the lower record is 0.12σ later, and is much more gradual (pre-alpha), than in the upper. The upper is simple; the lower gives evidence of beta and gamma in addition to pre-alpha and alpha. The marks on the lower base line indicate successive σ for both records. Similar, but very much lower, pictures are obtained when the roots are stimulated separately while leading from the nerve trunk. X = 85 mm. R = 2,000$^{\omega}$. Natural size.

taken separately from points on the ventral and dorsal roots equidistant from S_1, say through $E_m - G_m$ and $E_s - G_s$, respectively, the action potential appearing in the motor root always is simple (fig. 2, upper), while that in the sensory root always is compound and consists of alpha,

beta, gamma and, sometimes, delta waves (fig. 2, lower); it has the familiar appearance of the conducted action potential in the sciatic nerve. If the strength of the stimulus be now gradually reduced, at first the sensory action potential alone is altered, the delta (if present), gamma, and beta waves disappearing in succession in the order mentioned just as happens in the sciatic nerve. With further reduction in the strength of the stimulus the alpha sensory wave and the motor wave eventually diminish in amplitude and disappear, and this occurs in both roots roughly at the same level of stimulation. These results have been observed alike in preparations from the bullfrog, green frog and cat.

Similar results are obtained (observations in the bullfrog only) when the conduction, instead of being ascending from nerve trunk to roots, is descending, the stimuli being applied to the roots separately while leading from the side and end of the sciatic nerve distally; the action potentials, however, are lower in amplitude due to shunting by the large number of inactive fibers. Stimulation of the motor root at any sufficient strength gives only a simple action potential wave in the sciatic nerve. The stimulus that gives a just maximal motor wave is apt when applied to the sensory root to produce in the nerve an action wave that is simple also; but in this case upon increasing the strength of the stimulus beta and gamma, and sometimes delta, waves add themselves to the picture.

It should be pointed out that though the pattern of the sensory root action potential resembles closely that exhibited by the sciatic trunk, one nevertheless gains the impression that they are not exactly alike. Owing to the difficulty referred to in the section on methods it has not been possible, however, to get in one and the same preparation records that are exactly comparable. If it should turn out that the pictures are not quite alike the differences might be accounted for in several ways, such, for example, as unequal delays in the posterior root ganglion, or differences in the fiber distribution to the several roots, the sciatic pattern then being the result of the sum of the patterns contributed by each of its roots.

However this may be, there can be no doubt that the alpha, beta and gamma waves in the sensory root, like those in the sciatic nerve are due to group differences in irritability and conductivity of the component fibers. That this is the case we have been able to demonstrate by applying one of the tests used in our previous work (Erlanger, Gasser and Bishop, 1924) to prove that the waves represent action potentials in different groups of nerve fibers. Thus we have shown previously in the sciatic nerve that upon causing an ascending action wave consisting of the alpha and beta processes (both maximal) to pass through a descending action wave consisting of a maximal alpha process only, the alpha process of the former disappears, the record showing only a beta wave. Similarly, in the case of a root preparation containing the fibers from one pair of roots only (see

fig. 3), by causing an ascending action wave consisting of maximal alpha and beta processes (fig. 4, upper record) to pass through a descending maximal alpha process started by stimulation of the nerve trunk at a

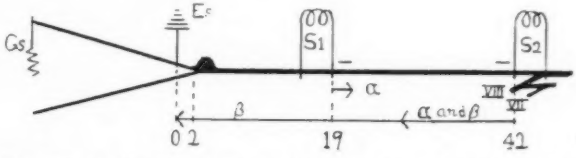

Fig. 3. Diagram of the connections on the nerve from which the records of figure 4 were obtained. A stimulus maximal for alpha was first applied through S_1 and one maximal for alpha and beta through S_2 while the alpha fibers at S_2 were still refractory. The lead was from the dorsal root at E_s.

somewhat more central point (at S_1), the action potential recorded in the sensory root is altered by the loss of its alpha wave (fig. 4, lower record).

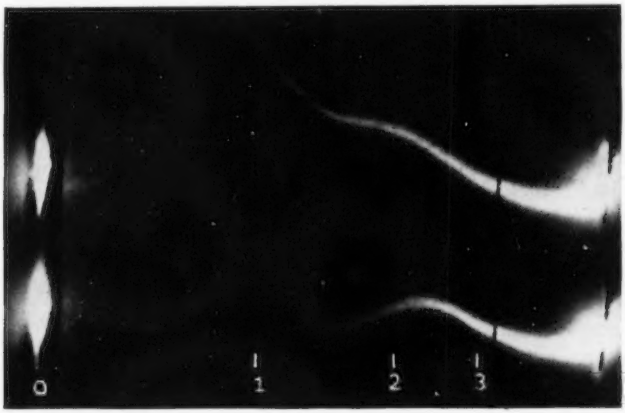

Fig. 4. Records from an experiment (4, 16, '25) demonstrating that the beta wave in the dorsal root (bullfrog, IX) is conveyed by fibers other than those conveying the alpha wave. The nerve was arranged as shown in figure 3. Upper record = the action potential from S_2 showing alpha and beta. Lower record = same as upper record except that an action wave descending from S_1 (fig. 3) has made the alpha fibers at S_2 refractory; beta alone, therefore, appears in the root. X = 86 mm. R = 2,000$^{\omega}$. The time in σ is indicated on the base line of the lower record. Natural size.

Occasionally the action potential wave appearing in the dorsal root in response to stimulation of the sciatic trunk appears to be made up of an additional component wave travelling in more irritable and faster fibers

than those determining the main alpha potential. In such instances, while carefully increasing the strength of stimulation, the growth of the alpha wave slows slightly and temporarily at the point where, we believe, the fibers of this more irritable group have all been stimulated; and in such instances the ascending phase of the full alpha wave may present an indistinct discontinuity shortly after its start, which might very well be attributed to the effect produced by a group of fibers conducting slightly more rapidly than the main alpha group (see, for example, fig. 2, lower record). This group of fibers, for the sake of convenience, we have designated *pre-alpha*.

Delay of the action wave in the spinal ganglion. In every preparation tested the alpha action wave started simultaneously in the sensory and motor fibers by stimulation of the nerve trunk or of the roots, after passing through the region of the dorsal root ganglion in either direction, has appeared at the lead later in the sensory than in the motor fibers. Sixteen quantitative determinations in the bullfrog's sciatic (see table 1) give delays which, with but one exception (6, 5, '25) vary between 0.09 and 0.18σ, the average being 0.135σ. If the 0.02σ delay be excluded the average becomes 0.143σ. Six determinations in three preparations from the green frog range between 0.04 and 0.14σ, with an average of 0.08σ. These were at room temperatures, which ranged between 22 and 27°C. One determination in the cat at a temperature of 35°C, and in oxygen, showed a delay of 0.08σ. These determinations are based upon the measurement of photographic records of the action waves (see fig. 2) and practically, their accuracy is limited by the accuracy with which the beginning of the action wave can be located on the record. This can usually be done within 1 mm.; and 1 mm. of a time line determined by charging the condenser through a resistance of 2000 ohms, and located where the start of the action wave usually stands in these experiments, subtends 0.026σ. The error of measurement in round numbers, therefore, is about $\pm 0.03\sigma$. With the one exception this error, it is seen, almost covers the range of the determinations around the mean values. The mean values must therefore represent fairly accurately the lag of the fastest of the sensory axon potential waves behind the fastest of the motor.

To demonstrate that after the action wave has passed the region of the ganglion it is later in the sensory than in the motor fibers is a comparatively simple matter. It is only necessary to stimulate the nerve trunk at any point and to lead from the roots separately, or to stimulate the roots separately while leading from the trunk, the conducting distances being always the same in comparable determinations. But to prove that the tardiness of the sensory action wave is due to a delay in the ganglion it is necessary to show that it develops neither in the nerve trunk nor in the sensory root. That the delay is not due to a conduction rate in the sen-

sory fibers that is slower than that in the motor fibers in the sciatic trunk can easily be demonstrated by determining the effect upon the duration of the lag, as measured in the roots, of changing the distance the action potential is conducted in the nerve trunk. The data collected in table 1 show quite clearly that irrespective of the position of the stimulating electrodes on the nerve the lag always is about the same within the limits of the experimental error. This is true also in the experiment (6,17,'25) in which the stimulus was applied separately to the roots while leading from the nerve trunk at two different distances from the ganglion.

It can also be shown that the sensory lag is not attributable to a difference of the rate of conduction in the two nerve roots, though in this case the demonstration cannot be made so convincingly because of the limited length of fiber available. It may be stated, however, that such observations as we have made (see table 1) show no consistent differences in the lag depending upon differences in the distance of conduction along the roots. Furthermore, any influence the roots might exert through differences in conduction rate is practically eliminated where the lead is from the crotch of the roots; and in such leads the lag, within the limits of the experimental error, is as large as in any other.

It is clear, therefore, that the sensory action wave is delayed in the posterior root ganglion, and that the delay is not due to slower conduction in the sensory than in the motor fibers. As a matter of fact we have some observations which seem to indicate that if there is any difference in the rates with which the motor and alpha sensory action waves are conducted it is in the direction of slightly quicker conduction by the fastest sensory fibers. The evidence, however, is rather fragmentary. In one experiment (12, 13, '24) the roots were separated until the crotch lay just distal to the spinal ganglion. In this case measurements made on the face of the oscillograph showed an exactly simultaneous arrival of the motor and pre-alpha sensory waves at lead E_c (in this case distal to the spinal ganglion). In another experiment in which a nerve was stimulated in the general position of S_1 while leading through $E_n - G_m$ (fig. 1), it was found that as the strength of the shock was gradually increased the first deflection to appear consisted of a small diphasic action wave. Soon, however, a large monophasic action wave appeared, and the beginning of the latter was distinctly later than that of the former. This picture undoubtedly signifies (1) that the threshold of the alpha sensory fibers was slightly below that of the motor fibers, the diphasic wave being the sensory action wave recorded through E_n as one lead and G_m, extended by the motor root, as explained above, as the other lead, the monophasic action wave being the motor effect recorded in the usual way. And the picture signifies also (2) that the sensory fibers conducted slightly more rapidly than the motor. Quantitative determinations were not made.

With this same preparation, however, the difference in the rates of propagation of motor and sensory waves was demonstrated in still another way and measured. The nerve was stimulated at S_1 (fig. 1) with induction shocks that were maximal for the motor and the alpha sensory, but below the threshold of the remainder of the sensory fibers, and the action wave was obtained by connecting with the amplifier through $E_n - G_m$. Then, while observing the position of this action wave upon the fluorescent screen, the sensory root was stimulated in an efferent direction with strong induction shocks so timed that the action wave ascending from S_1 passed through the action wave descending from the sensory root and reached E_n after the descending action wave had cleared that point. In this way the record from E_n was changed from one produced by all of the axon action waves ascending the nerve into one determined by the motor constituents only. The ascending action wave very definitely moved to a slightly later position on the fluorescent screen each time the descending stimulation was begun. Photographic records show a time difference of 0.037σ. This difference is almost within the limit of the error of measurement and would be given little consideration were it not for the very obvious shift that could be observed over and over again on the face of the tube.

Another experiment by still another method failed to reveal any difference in the rate of conduction of the fastest of the motor and sensory axon waves. In this case three pairs of stimulators were used. One on each of the roots at the same distance from the crotch and connected through a double throw switch with the same inductorium, and a third on the nerve a short distance distal to the ganglion and connected with a second inductorium. The cathodes of all stimulators were distal and the lead was from the side and end of the nerve distally. The sensory root was first stimulated with a shock that was just maximal for alpha and then, after the resulting action wave had passed the stimulator on the nerve (observed by leading temporarily from the stimulator), but while the fibers that had responded were still relatively refractory at that point, the nerve was stimulated through the lower stimulator with a shock which again was maximal for the alpha fibers that had not conducted the action wave from above, but below the threshold of the fibers which had been active. Under these circumstances only the motor fibers could respond to the stimulation of the nerve trunk. Then, by means of a similar procedure, in which, however, the motor root was stimulated instead of the sensory root, a record of the sensory action wave was obtained. Comparison of the two records thus obtained showed absolutely no difference in the times of arrival of the two action waves, motor and sensory, at the end of the nerve.

The foregoing experiments, therefore, prove that the fastest sensory axon conducts at least as rapidly as the fastest motor, and indicate that

the lag of the sensory waves in the ganglion as determined by our experiments is to be taken as a minimum figure. These experiments indicate also that slight differences in the relative rates of these two fibers may occur from nerve to nerve.

Reference should be made here to the experiment (6, 5, '25) which shows the lag of only 0.02σ. Even if to this value were added the time, 0.04σ, the sensory action wave in the same preparation gained on the motor action wave in the nerve trunk, the divergence from the mean of all the lags would still be greater than the experimental error. Under the circumstances it seems necessary to invoke slowed conduction in the motor root resulting from damage, to explain the lower value in this case. This interpretation receives support from the fact that when the distance of conduction in the root was shortened to a minimum by leading from the crotch, a much longer lag, 0.09σ, though still the shortest of the series, resulted.

TABLE 2

Data on the relative irritabilities of the motor and alpha sensory fibers

11, 19, '24.	Green frog. Assigning a value of 100 to the primary amperage that is threshold for the most irritable fiber, the values obtained were as follows: threshold for motor and pre-alpha sensory 100; alpha sensory 115; just maximum for motor and alpha sensory, 185 and 162, respectively.
11, 22, '24.	Bullfrog. Sensory irritability slightly greater than that of motor.
1, 7, '25.	Green frog. Pre-alpha sensory a trifle more irritable than motor.
6, 5, '25.	Bullfrog. Sensory slightly more irritable than motor.

The relative irritabilities of the alpha groups of fibers. In a few of the experiments the relative irritabilities of the motor and of the sensory components were noted. The leads, as usual, were taken from the motor and sensory roots separately. Since the stimuli were applied to the nerve trunk we may be sure that all comparable stimuli were identical, barring possible differences due to the position of the fibers in the trunk relative to the stimulating electrode. The strength of the stimulus was varied by means of a slide wire resistance in series in the primary circuit. The information thus collected, which, in the main, is tabulated in table 2, is rather fragmentary, but such as we have justifies the statement that if there is any difference in the irritability of the most irritable of the motor and of the sensory fibers it is very slight and in the direction of a higher irritability on the part of the latter or, at least, of the pre-alpha group of fibers. This result is consistent with our observation that the fastest of the sensory axons may at times conduct slightly more rapidly than the fastest of the motor axons. The one quantitative experiment (11, 19, '24) indicates that the least irritable motor fiber (measured by the just maximal stimulus) is somewhat less irritable than the least irritable alpha sensory

fiber. Due, however, to the complication, in the case of the sensory root, caused by the entrance of the beta wave at about the time the stimulation is becoming maximal for the alpha process, the just maximal stimulus of the alpha sensory process cannot be determined as sharply as in the case of the motor wave. We do not, therefore, care to lay any stress upon the comparatively small difference noted in this single observation.

Comparison of motor and alpha sensory axon action waves. The duration of the action wave obtained by leading from the nerve at or close to the site of stimulation when the stimulus is a brief induction shock is the duration of the contributing axon action waves (Erlanger, Bishop and Gasser, in press). In one preparation leads were taken 3 and 4 mm. removed from the stimulating electrodes on the sensory and motor roots, respectively. Durations of 1.92 and 1.86σ were obtained. These may be regarded as alike within the limit of error. In another preparation a record obtained from the sensory root by leading directly from the site of stimulation gave a duration of 1.50σ. By extrapolation of the durations of the action wave at two distances of conduction, in another experiment (5, 14, '25), the motor action wave at zero distance of conduction was found to have a duration of 1.62σ. The motor and the alpha sensory axon action potential waves have, therefore, essentially the same durations.

The range of rates of the axon waves in the motor fibers can be determined roughly from a, the lag of the slowest fiber, obtained by subtracting the duration of the axon action wave (derived by the above-mentioned extrapolation) from the duration of the action wave conducted a known distance, and b, the conduction rate in the fastest fiber. One experiment of this kind shows that the slowest fiber was conducting at the rate of 29 m.p.s., the rate of the fastest fiber at the same time being 42 m.p.s. (bullfrog).

The determination of the range of rates in the alpha sensory wave by this method is not so simple. Thus, to use this method, it is necessary to assume that all the axon waves are delayed equally in the ganglion, and we have no definite evidence that such is the case. And again, owing often to absence of a margin between the maximum stimulus for alpha and the threshold for beta, the duration of the alpha sensory process after conduction cannot often be directly determined. The results that have been obtained have, therefore, been quite variable. We have records of the conducted alpha sensory action wave started by a stimulus which was adjusted as carefully as possible for what was supposed to be just maximal for alpha, some of which are longer, others shorter than the corresponding motor action wave. The time to maximum of the waves can be measured much more accurately than wave duration. These measurements are included in table 1 where it can be seen that the times to maximum of the motor and of the alpha sensory waves obtained in response to stimuli supposedly just maximal for the alpha sensory process, likewise do not

bear a consistent relation to each other. Upon the whole, however, one gains the impression that the spans of rates in the alpha sensory fibers and in the motor fibers are roughly alike, but that the relation may vary somewhat from root pair to root pair.

Discussion. That the action potential passing from the nerve trunk into the ventral root is simple, while that travelling into the dorsal root is compound, when one takes into consideration the histological and functional composition of the two roots, is not surprising. The motor root to be sure contains two types of fibers easily distinguishable histologically, namely, the larger somatic motor fibers and smaller fibers passing into the sympathetic outflow. But only the former group contributes to the action waves started and recorded as in our experiments, and from fibers presenting so uniform an appearance as do these, one would naturally expect to obtain an action wave more or less simple in form.

In the sensory root, on the other hand, there is a great variety of fibers. It contains large numbers of unmyelinated fibers along with the myelinated, and the latter, furthermore, differ markedly among themselves in respect to size. If conduction rate and irritability are related in any way to fiber size and to fiber histology, the composition of the dorsal root certainly supplies a basis for a separation out of waves during propagation of the axon potentials along the fibers which enter the root.

Neither is it surprising, when one considers the functions mediated by the roots, that the action wave in the motor root proves to be simple, in the sensory root compound. The somatic efferent fibers have only one function to perform, namely, that of supplying impulses to voluntary cross-striated muscle. There is, therefore, no reason for expecting to find among the motor fibers group variations in irritability and in conductivity. On the other hand, physiologists have found it convenient, if not necessary, to classify the impulses carried by the fibers of the posterior root. Among such classifications may be mentioned those based on general distribution, for example, somatic and visceral, those based on general function, for example, exteroceptive and proprioceptive, and those based on specific function, such as the various senses served, etc. Our work has not progressed far enough to justify an attempt to fit the picture presented by the action potential into any structural or functional classification of nerve, though the constancy of the action potential picture of any given nerve convinces us that the waves have a very definite meaning of some kind. A word may, however, be said with regard to one phase of this problem.

In the sciatic potential record we can distinguish some three or four groups of fibers, namely, alpha, beta, gamma and delta, on the basis of irritability and rate of conduction. The present experiments indicate the necessity of regarding the alpha wave in the sciatic nerve as composed of at least two elements, namely, motor and alpha sensory. These two groups

are, however, so very nearly alike in physiological characteristics, in irritability and in conductivity, that when they are simultaneously stimulated the resulting action waves, motor and alpha sensory, merge indistinguishably to form a single wave apparently differing from either one alone mainly, if not only, in respect to height. The action potential in the phrenic nerve on the other hand is simple. Since it is well known (Sherrington, 1894) that the phrenic contains an abundance of sensory fibers along with the motor, it becomes clear that the action potential wave in the phrenic, like the first wave of the sciatic, is composed of two completely superimposed elements, one motor, the other sensory. In a previous paper (Erlanger, Bishop and Gasser, in press) we have shown that the range of conduction rates in the alpha fibers of the sciatic nerve is relatively the same as in the phrenic fibers, a fact which can be regarded as further presumptive evidence in favor of the functional homology of the alpha sciatic and phrenic waves. Since the phrenic fibers mediate mainly motor and muscle sense impulses, it seems highly probable that the alpha sensory fibers in the sciatic likewise convey the muscle sense impulses.

Before discussing the problem raised by the finding of a lag in the passage of the action potential wave through the posterior root ganglion it is necessary to refer to an apparent contradiction between this result and the conclusion reached by Lenninger (1913) relative to the same question. Lenninger, on the basis of experiments similar to ours, but done with the string galvanometer instead of the cathode ray oscillograph, has concluded that the arrival of the action potential in the ventral and dorsal roots occurs without any appreciable lag in the spinal ganglion. Examination of Lenninger's data shows, however, that he reached this conclusion as a result of overcaution in the interpretation of his data, and that, as a matter of fact, his and our determinations agree remarkably closely.

In 12 determinations made on one bullfrog preparation Lenninger records lags ranging from zero (2 cases) to 0.35σ, the average of all of these being 0.12σ. In 3 other experiments his results are expressed in terms of conduction rate in the motor and sensory fibers. From these data the lag can, however, be calculated by arbitrarily assigning a conducting distance. If this be put at 80 mm., the longer distance, approximately, in the one of his experiments that gives this information, it turns out that in three of the six determinations there was a zero sensory lag and in the remaining three, lags amounting to 0.23, 0.32 and 0.38σ, the average of these six determinations being 0.15σ. The only difference between these results and ours, therefore, is that our range of variation is narrower (his, zero to 0.38σ, ours, 0.09 to 0.18σ), the average of all of his lags being, however, 0.13, of ours 0.14σ.

The close agreement between the results of these two sets of observations obtained through the use of wholly different methods, leaves no room for

doubting that the sensory impulse is delayed in the posterior root ganglion, and we may, therefore, turn next to a consideration of the cause of this delay. The natural inclination is to attribute it to the passage of the impulse up and back through the process of the ganglion cells, plus, possibly, a delay in the cell. To and fro conduction alone, if it be at the same rate as in the axon, and even if an extra distance of conduction of 1 mm. be added to a straight distance of 1 mm. in order to care for the spiral and tortuous course of some of the cell processes such as Cajal (1906), Dogiel (1908) and others depict, would suffice to account for not more than half of the lag we have found. There is little reason for believing, however, that this detour is made. For, in the first place, experimental evidence, as is well known, is against it. Thus Bethe (1898) has shown, in *carcinus*, that after cutting through the stems of the ganglion cells in such a way as to leave none of the cell bodies in connection with the extensions from the cells to the antenna, the tonic and reflex activities of the antenna remain intact for a period of 24 hours. Bethe very guardedly concludes from this observation that the "nucleus bearing part of the neuron" is not needed for the exhibition of these functions. The reason for his putting the conclusion in this way undoubtedly is to be found in the architecture of these cells in arthropoda. They give rise to "a single immense branch" (Edinger and Hall, 1900), which, after a short course, gives off the two fibers. Bethe evidently regards the process as a part of the cell body rather than as a nerve fiber. It seems, therefore, that there may be some question with regard to the applicability of Bethe's conclusion to cells having the structure of vertebrate spinal ganglion cells.

Additional evidence against the passage of the impulse up to the spinal ganglion cell has been presented by Steinach (1899), who has shown that in excised preparations consisting of sciatic nerve and dorsal roots and in preparations of the sciatic and dorsal roots left *in situ*, but deprived of their blood supply, the functional activity of the nerve, as tested in the former case by the negative variation, and in the latter, by reflex activity, persists after the cells of the spinal ganglion present "undoubted" histological evidence of degeneration. Steinach, therefore, concludes that centripetal conduction is characterized by its complete independence of the ganglion cell.

A much more serious difficulty in the way of a to-and-fro passage of the impulse through the cell stem is its refractoriness. The cell stem to all appearances is an axon. If it conducts as does an axon, the passage of the impulse to the cell would leave the stem in a refractory state, which would block return passage of the impulse for a period lasting about ten times as long as the observed lag of the impulse in the ganglion.

The possibility has to be considered in this connection of the lag being the result of asphyxial slowing of conduction in the fibers embedded in the

ganglion. There is little to be found, though, in support of this idea. It is inconsistent with the uniformity of the lag in any given species, and with its constancy over comparatively long periods of time in successful experiments. Furthermore, if the lag were due to slowed conduction, the rate in the fibers within the ganglion, which in the bullfrog is about 4 mm. in length, would have to be 17 m.p.s., taking 42 m.p.s. as the normal. A rate of conduction of alpha as slow as this we never have seen even under the very worst of conditions.

The only normal basis for a delay that occurs to us is some hindrance to the passage of the potential wave through the point where the centripetal (with respect to the spinal ganglion) branch joins the centrifugal branch, and evidence is lacking for or against such a view. If, however, there were a delay at this point, one would not expect it to be of the same order of magnitude as the synapse delay. As a matter of fact the lag we find (0.14σ) is less than $\frac{1}{100}$ of that believed to occur at the spinal synapse in the frog (Buchanan, 1908).

SUMMARY

Previous work has shown that the action wave in the sciatic nerve, recorded, after amplification, by the cathode ray oscillograph, breaks up, during conduction, into four components which have been designated *alpha*, *beta*, *gamma* and *delta* in the order of the position they take in the nerve due to differences in rates at which they are propagated.

Employing similar methods it is now shown that the quickest component, alpha, itself is compound consisting in reality of the sole *motor* component, which passes into the ventral spinal root, and an *alpha sensory* component which passes into the dorsal root. The fibers contributing to these two waves have about the same stimulation thresholds and rates of conduction and consequently the two waves are indistinguishably merged in the artificially stimulated sciatic nerve.

It is inferred that the simple action potential wave of the phrenic nerve likewise is composed of two waves, the motor and the alpha sensory, indistinguishably merged.

The fact that the phrenic nerve is made up almost exclusively of motor and muscle sensory fibers suggests that a part at least, of the alpha sensory fibers in the sciatic nerve have to do with muscle sense.

The beta, gamma and delta sciatic waves are entirely sensory, since, like the alpha sensory wave, they pass into the dorsal root. In addition a small *pre-alpha sensory* wave has occasionally been seen; its stimulation threshold and rate of conduction place it very slightly in advance of the alpha sensory wave.

In the bullfrog the alpha sensory action wave falls behind the motor action wave 0.14σ (average) during its passage through the spinal ganglion.

In the green frog the delay averages 0.08σ; and in one determination in the cat it was 0.08σ. The delay is greater than the time an impulse travelling at the normal rate would require to make the detour into the ganglion cell. No decision is reached with regard to the cause of the delay.

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DISTORTION OF ACTION POTENTIALS AS RECORDED FROM THE NERVE SURFACE

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Incidental to the study of nerve action potentials by means of the cathode ray oscillograph, various artefacts have come to light that need be taken into account in the interpretation of the wave form, area, or time of critical events in the excitatory process. These artefacts depend only in part upon the apparatus, and are also in part a result of the structure of the nerve trunk as employed, or even of the nerve axon itself, and thus may be in a way physiological. Several of them are associated with the presence of a connective tissue sheath about the nerve, which alters, by its polarization, the time-strength characteristic of any current which passes through it. They affect therefore both the apparent irritability and the apparent reaction of nerve as recorded, and since polarizations of this sheath and of the axons themselves are presumably similar processes, the understanding of these distortions is pertinent to the precise interpretation of the essential reactivity of nerve. Certain of these artefacts have been described as they were detected in previous papers. Distortions due entirely to the type of apparatus used for recording (amplifier and cathode ray oscillograph) have been so discussed, and will not be dealt with here in any detail. They are relatively insignificant so far as discovered, and may be either corrected or allowed for by calibration, etc.

The distortions to be discussed below, however, affecting the accuracy of observation by means of any apparatus which is sufficiently sensitive to record them, are of more general interest. These may be summarized briefly as physical distortions, due to such factors as capacity, resistance, and leaks in the stimulating or leading off circuits; and physiological distortions, due to physiological or mechanical condition of the nerve, such as extent of killed area, effect of dead fibers in a trunk, effect of cut branches between leads, and virtual double leads of various kinds, etc. The two types, physical and physiological, are really not at all distinct, and any classification is arbitrary. Certain artefacts or distortions, while due in part to mechanical arrangements in the apparatus, still include the effects of physiological activities in the nerve; for instance, the so-called escape of stimulus is in part at least an effect of the nerve's local reaction to

stimulation; the nerve reacts by polarizing. The only previous analysis of the escape with which we are familiar is that of Forbes and Gregg, in which the distribution of capacity in the recording instrument and its relation to the distributed capacity in the nerve was considered. While admitting the possibility of this source of disturbance in the circuit used by Forbes and Gregg, we do not find it to be of appreciable magnitude in our own experiments, presumably because our input is of much lower capacity than that of a string galvanometer. In addition to distributed capacity there is a second and we believe much more important factor acting in the same way, polarizable resistance, both in the nerve axon and in the connective tissue nerve sheath. We have accordingly included under the latter term, polarizable resistance, that part of the nerve's reactance which can be balanced out by a capacity in series with a damping resistance in the opposing arm of a Wheatstone bridge. This only implies that a part of the nerve's reactance can *be treated as* capacity damped by resistance. The rest of the resistance (nonpolarizable), can be balanced out by metallic resistance. The nerve's polarizable resistance is presumably a function of semipermeable membranes acting as leaky "electrolytic condensers."

We believe that some of Forbes and Gregg's difficulties in interpreting odd escape forms may be due to overlooking the fact that two or more "capacities" in the circuit may be affected by different damping resistances, and therefore sum or superimpose to give complicated wave forms, since they have different time relationships. (See fig. 15, Forbes and Gregg, 1915.) With a visible record such as ours that can be observed as the circuit is manipulated, and which will record accurately, transient effects down to a period at least as short as 100,000 per second, we have been able to pick out and eliminate the separate components of these effects, one by one. A recent paper by Davis and Brunswick discusses certain of the phenomena described below as they appear in string galvanometer records.

a. The stimulus "escape." In a record made through electrodes anywhere on the nerve, the time of the start of the potential record due to the shock is the same as the time of the shock itself, that is, the nerve is affected at all points instantaneously. The sign of the potential change is that of the stimulating electrode proximal to the lead. The circuit traversed by the current seems to be the typical core conductor circuit, with the galvanometer leads at two positions on the path along which the shock potential falls. The duration of the "escape" is longer however, than the duration of the shock, often much longer. The shape of the potential curve of the escape furthermore may be quite different from that of the shock potential itself. In other words, the escape is not a mere record of the shock, but is also dependent on the properties of the nerve.

This is further indicated when attempts are made to balance out this escape, in order to obtain records of the undistorted nerve action potential close to the stimulated point. It can then be demonstrated that the escape as recorded is a composite effect of at least three separate factors; 1, the polarization of the nerve by the shock; 2, the reactance of the secondary or stimulating circuit, and 3, the direct electrostatic field distortion from the break of the primary coil current, affecting the secondary and nerve circuit either through capacitative coupling with the secondary coil, or through the air, from primary to amplifier input leads. A ground connection in the primary is not essential to this effect.

The distributed capacity of the secondary circuit, largely confined to the coil, is probably effective chiefly because it is asymmetrically located in the circuit with respect to the source of potential. It affects the form of the potential discharge through the nerve, and thus the escape. The coil thus acts as if part of its resistance were shunted by capacity, at one side of the potential source. The galvanometer leads, though actually taken from the nerve below, are in effect leads from anode and cathode of the stimulating circuit, down the nerve through the inside and outside of the core conductor respectively. They have different resistances, and these are polarizable, and a polarizable leak connects outside and inside all along the nerve from stimulator to leads. Thus the shock, of whatever form, impressed upon the nerve, is further distorted in the shunt circuit to the galvanometer. The coil capacity may affect the record because it is involved in this circuit. Since the capacity of our input is negligible, the direction of the shock and its distortion due to the coil capacity depend almost entirely on the coil connections. The direction of the unbalanced cathode-proximal coil shock escape is always negative, the same as the nerve potential record; but if it is very slight, the primary escape may reverse the record of the secondary escape. More complicated distortions of the secondary escape depend upon the details of the circuit.

If the nerve is replaced by a metallic model, of the same resistance distribution as the nerve, (fig. 1) the escape disappears; there is no polarization, and the amplifier input is grounded through the "nerve" *dc* and the lead *c*. A high resistance (R4) in *c* restores the primary effect, but the secondary is short circuited through *ab* and does not appear, unless a second ground is placed at some point of the secondary circuit, say *a*. The "escape" is then (disregarding the slight primary effect) an accurate record of the change of potential at the secondary terminals.

If in place of the metallic resistances a piece of yarn wet with salt solution is mounted on platinum electrodes, there is no appreciable escape without the ground at *a*, and with this ground, the record of the secondary potential is distorted by polarization at the electrodes insofar as this occurs. The condition is still not that of nerve, for nerve gives the

polarization escape without the ground at *a*. The difference is apparently in the structure of the conductor; the nerve axons are surrounded by myelin sheaths, presumably polarizable, and the whole trunk by a connective tissue sheath, also polarizable. The salt bridge is polarizable at the electrode surface, but this surface polarization effect is shunted out by the low and non-polarizable cross resistance of the salt solution. Apparently in the nerve on the other hand, the recorded potential is a potential drop along the nerve trunk surface, or axon surfaces, which decreases as one passes away from the stimulator by leakage of current through the connective tissue sheath, or through the axon sheaths. This leakage current is presumably conducted back through a low resistance core, past the electrode from which is flowed, (proximal to the lead) to the other (distal) stimulating electrode.

The escape as recorded thus includes polarization of the nerve, the immediate and direct electrochemical effect of the electrical shock, and though this polarization is by no means a true physiological response to

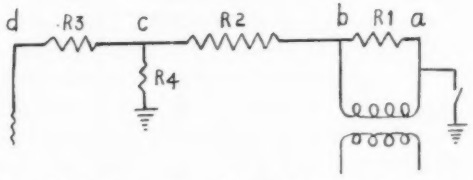

Fig. 1. Metallic nerve model. For explanation see text

stimulation, even locally, as the action potential wave which follows it is, it includes axon polarization, which may be the effective stimulus to nerve. It varies directly with the strength of the applied shock.

With a commutator switch placed in the primary circuit and both secondary leads either opened or short-circuited, reversal of the primary current has no effect upon the amplitude or sign of the primary "escape" as recorded on our apparatus. Changing the angle between the axes of primary and secondary coils, thereby modifying both their electrostatic and magnetic coupling, has no appreciable effect. Altering the strength of the primary current has an effect. Opening the primary circuit at one side of the coil, with the commutator key closed and the interruptor running, abolishes the primary escape. This is due to the fact that the self-inductance of the primary coil, upon the break, is the source of the charge on the primary leads which act as one plate of a condenser, of which the amplifier input is the other plate. The effect is thus an electrostatic one, through the air as a dielectric, which is but little augumented by further capacitative coupling between the primary and the secondary coils, with a lead through the nerve to the amplifier input. It is so small

an effect that the secondary escape generally overpowers it, though the sum of the two, when in opposite directions on the record, may give a complicated picture that simulates an oscillation. It is convenient as a record of the time of the shock and is of so short duration (0.000,02 second), that it does not seriously interfere with an action potential record.

The combined effect of secondary reactance and nerve polarization can be roughly compensated by putting the nerve in one arm of a Wheatstone bridge, in the opposite arm of which is placed either a variable polarizable resistance, in series with a variable metallic resistance, or a capacity with both shunt and series resistance. Since the two components of this "escape," nerve polarization and circuit reactance, have different time relations, any balance of this bridge will leave a residual effect of one or the other distortion, though this residuum can generally

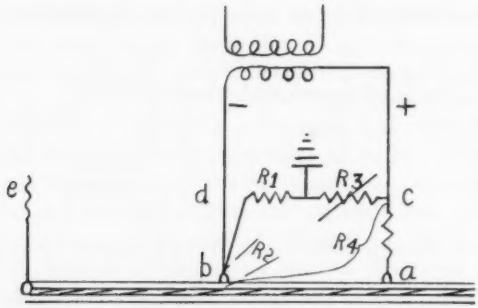

Fig. 2. Bridge arrangement of secondary circuit to balance out escape due to polarization and capacity. For explanation see text. R_1 electrode resistance, R_2 nerve resistance under electrode (transverse), R_3, variable resistance for balancing, R_4, electrode and nerve resistance.

be reduced to values that will not interfere with the records. The logical treatment would be to place a wave trap consisting of 2 or more series-condenser-and-resistance units in parallel, in the bridge arm; but for each position on each nerve these would have to be readjusted, and so far this complication has been avoided. It is more satisfactory to use one variable resistance in series with fixed capacity of approximately correct value, and shunt the whole with a second variable resistance; and then so handle the nerve that its "escape" will be a minimum.

An arrangement such as figure 2, makes a virtual bridge of the nerve circuit, and leaves the secondary coil itself out of the bridge proper, as the source of the applied potential. *a-b* are two platinum electrodes, *c-d* the terminals of the coil. R_1 is the resistance of a calomel electrode,

which besides being one arm of the bridge, is the effective ground lead for the action current. R_2 is merely the resistance of the connective tissue sheath at b, and is polarizable. R_3 is a variable graphite line resistance from anode of coil to ground. R_4 is the resistance of the nerve and of the sheath at a, which is also polarizable, plus that of the electrode at a. Its polarization is roughly balanced by that of R_2, though its resistance is of the order of R_3, by which the bridge is balanced for resistance. Some point inside the sheath can thus be maintained at ground potential, and when this point is that which has the same potential as the grid lead e, when current is flowing, the action potential will be recorded, but the shock will not.

The effectiveness of this circuit depends upon an accurate balance between the polarization potentials at a and b, and this is not usually very perfect. It is found however, that if the nerve's connective tissue sheath is dissected off, or if the spinal root is employed, which has no heavy sheath, the distortion by the stimulus is reduced enormously, even without the bridge arrangement, and with this, can generally be rendered quite innocuous. So striking is the effect of sheath absence that it must be inferred to be the locus of a large part of the polarization phenomena of the nerve trunk as usually observed.

It is apparent that reversing the current through the primary will reverse the secondary and polarization effects in the "escape." It is also obvious that the further one leads from the stimulator, along the nerve, the lower will be the escape due to the secondary current, until at long leads, the primary effect may predominate. If the secondary resistance is sufficiently high to cause the coil circuit to oscillate, the oscillations, readily observable on leading from the coil terminals directly into the amplifier, or from the cathode on the nerve in an imperfectly balanced bridge, disappear from the record of the escape as it appears without balancing the circuit, leading at a distance from the cathode. The escape curve follows closely the curve of decay of the oscillations, the individual oscillation of potential being of too short duration to depolarize the nerve at each reversal. This is further evidence that the escape consists in part of the polarization effect of current flow, rather than presenting a record of the shock itself. Comparing a direct picture of the secondary shock, oscillating or logarithmic, with the "escape," the escape is seen to rise more slowly, with its maximum well after the maximum of the applied potential, exactly as should be expected in a polarization effect from a transient current. Forms of the escape as we have observed them are analysed graphically in figure 3.

If the "escape" record is a polarization effect, it should decrease in intensity at a distance from the point of application of the stimulus quite as any other polarization effect does. We have measured the decay of

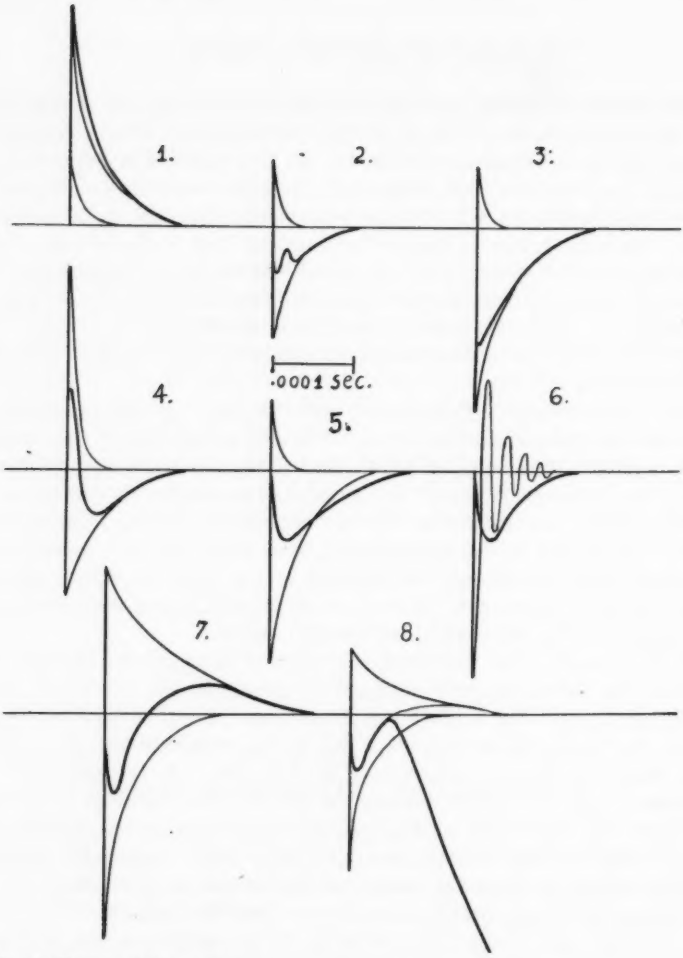

Fig. 3. Forms of the escape.

1. Short low primary and long anodal secondary shock summed.

2. Primary and low cathodal shock summed.

3. Primary and high cathodal shock summed.

4. Short high primary and long secondary cathodal shock, as recorded on oscillograph.

5. Low primary and long high cathodal secondary, and resultant polarization.

6. High resistance secondary oscillation (primary ignored), and resultant polarization escape.

7. Cathode secondary escape (primary ignored), and long lasting effect in opposite direction, of unknown origin.

8. Stimulating electrodes in bridge, with an approximate balancing out of two escape distortions in opposite directions, by one capacity. The result is a summed low distortion. The action wave rises from this low escape as a base line. This secondary escape is much smaller on a nerve without a sheath, allowing a balance of the bridge that approximates no escape.

electrotonus along the nerve due to application of constant potentials to a given region. At *a-b* (fig. 4) potentials were applied to a nerve of uniform size without branches, (bullfrog sciatic, region in and above thigh), and led off near the other end, from *d*, and from *c*, a movable electrode. The demarcation potential for any given position of *c* was read on a potentiometer to 0.01 mv., and subtracted from the electrotonic potential when current flowed through *ab*, for various applied potentials of from 50 to 500 mv The potentials at *c* plotted against the distance of *c* from *b* all fall precisely on an exponential curve of the form $b.y = 2^{-\frac{x}{2}}$, in

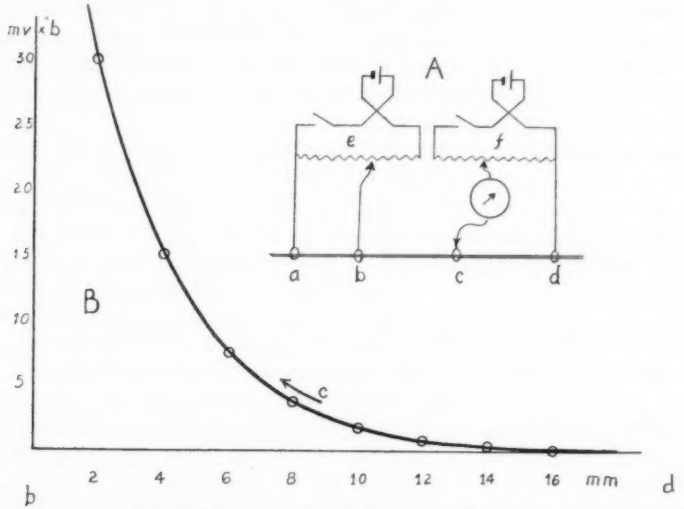

Fig. 4. A. Arrangement for electrotonus measurement. For explanation see text. B. Curve resultant from above measurements. For explanation, see text.

which *b* is the proportionality factor reducing the potentials to a common value, and *x* is measured in mm. along the nerve. This formula states that the polarization potential value at any point *c* which is 2 mm. further from the point of application of the potential than another point *c'*, will be one half the value of the polarization potential at *c'*. The intensity of the "escape" as observed along the nerve falls off considerably less rapidly than this. The polarization component of the escape presumably would follow this curve. The discrepancy is not readily explained, and may indicate either that there is a fourth component of the escape not enumerated above, or that the effect of the transient shock is quite different from the effect as expressed in the equation for the steady current.

b. Oscillating shocks. In certain experiments, to prevent the secondary of one coil from acting too extensively as a shunt to that of another, when both were connected in parallel with the same electrodes, we have loaded the secondary with a high resistance, increasing the strength of primary currents correspondingly. With a resistance of about 100,000ω, the coils we use, (Porter coils with iron cores removed), oscillate into the capacity of their own secondary windings. The period of these oscillations may be of the order of 0.000,01 second. A current oscillating continuously at this frequency would probably not ordinarily stimulate, certainly not if of the voltage used in these experiments. However, these oscillating shocks do stimulate quite satisfactorily, in fact no difference could be observed in the stimulation value of oscillating and non-oscillating shocks which gave approximately the same general amplitude of escape, even though the oscillations were observed on the record to extend far to either side of the base line.

The explanation is undoubtedly that, though the discharge through the nerve is oscillating, the oscillation is that of a decrementing potential, and the algebraic sum total of all the oscillations gives a value approximating that of the usual logarithmic discharge from the coil. The first half of one complete oscillation is in the same direction as the non-oscillating shock, and since the oscillations are decrementing, the second half, in the reverse direction, fails to quite annul the unidirectional effect of the first half. These differences for each cycle of the oscillating current sum in one direction, and thus accomplish the same effect in the nerve as the non-oscillating shock. The phenomena are modified as should be expected by changes of capacity or resistance in the secondary.

c. Escape in galvanic stimulation. When galvanic current is used to stimulate, and the nerve balanced in a Wheatstone bridge by resistance and capacity so that the current applied does not show as a shift of the base line of the recording mechanism (Bishop, Erlanger and Gasser) there is always left, on the record, a transient deflection at make and break of the current. This serves as an essential record of the event, and is usually so short and so low as not to interfere with the measurements of the action potential. By elaborate wave traps this could doubtless be eliminated, but for obvious reasons, aside from added complexity of the apparatus, this is not desirable. It is due to the following conditions. The two leads from battery and circuit breaker to the bridge have distributed capacity, and the two halves of the bridge have quite different orders of resistance, one half, (cathodal) being very low to serve as a nerve lead at the site of stimulation, the other being very high to prevent recording at the anode. At each make and break of contact between these leads, their capacity charges or discharges through the corresponding arms of the bridge to the ground of the apparatus on the one hand and

through the nerve on the other. The time relations of these discharges are quite different from those of the nerve's polarization, thus the capacities across the bridge arms to balance the latter do not balance the former. When hard rubber half segments were used on the circuit breaker wheel as dead segments, frictional effects between rubber and contact spring further complicated the picture. In the present model such segments have been replaced by insulated bronze sections.

d. Extra idle electrodes as leads. When more than one stimulating or lead-off electrode makes contact with a nerve, the second (between the recording ground electrode and the stimulus) may act to give a virtual ground lead, allowing a record of the action wave as it passes. The effect is more pronounced if the ground lead used for recording has a high resistance. This effect may be due either to electrode capacity or a resistance leak to ground. In a water saturated chamber, the rubber tubing covering the lead wires may be sealed over the glass electrode at the point of emergence of the platinum wire with de Khotinsky's cement.

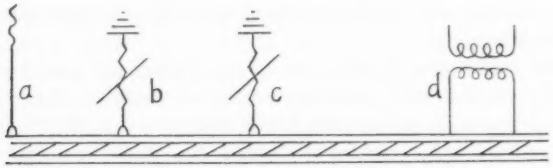

Fig. 5. Effect of doubleleads on nerve. For explanation, see text

The glass surface of the electrode itself apparently does not leak appreciably if kept free from salt solution. We have occasionally got apparent leads from the site of stimulation itself either into the capacity of the secondary coil, or through a leak in the stimulating circuit, due to a high resistance in the ground lead to the amplifier.

e. Double leads. When the extra idle lead is between the grid and ground (the two leads to the galvanometer), the results are much more serious. (Discussed by Davis and Brunswick.) In this case the capacity effect apparently is reënforced by a second factor, conduction of current along the layer of salt solution outside the nerve, from the extra electrode back to the ground lead. The result is a repetition of the action potential recorded from the ground lead, as the wave passes the interposed electrode. The second record may be nearly as large as the first and might easily be mistaken for a repetitive process, or for a second or β wave, depending on its time relationships. It may be conclusively distinguished from these however by observation of the result of altering the strength of stimulation. If the process is a β wave or a repetitive process it will disappear when the stimulus is reduced before the main process drops out.

If it is due to the extra electrode, the two waves change in exact proportion to their amplitudes, and drop out together.

That this is not the effect of capacity merely may be demonstrated by replacing the extra electrode by an insulated wet camel's hair brush, or even by a good sized bead of salt solution, when the same phenomenon may be observed. A mass of undissected tissue will produce a like effect.

In investigating this phenomenon, we grounded two electrodes from a nerve (fig. 5) and then varied the resistance in each ground separately. The action wave recorded at the electrode nearest the grid electrode, *b*, appeared alone whenever the resistance at that electrode was small, but a second wave appeared from the more distal lead, *c*, as the resistance increased in the proximal lead. The later wave, however, (at *b*) did not disappear even when the circuit to the *b* lead was opened, and did not change in position throughout the experiment. The late wave, from the free electrode between leads, *b*, is thus the limiting case of a lead from the electrode through a high resistance, and as stated previously, is not due to capacity of the free electrode. The high resistance circuit is obviously from the free electrode, along the wet surface of the nerve, to the grounded electrode.

That this is the case is still more obvious when we consider the effect of dissecting off the nerve's connective tissue sheath. The dog phrenic can be so dissected, or a frog nerve with its sheathless roots may be employed. If the region at which the nerve emerges from the sheath be located between the leads to the apparatus, a second action wave follows that recorded under the lead, at a time indicating that its point of registration is exactly where the nerve so emerges. If the ground electrode is moved to this point the waves superimpose without increasing in amplitude; if the electrode is moved away, toward the stimulus, the first wave appears earlier, (under the electrode) the second remains stationary and reduces in amplitude as the resistance increases along the nerve.

Now if even a wet contact on the nerve without appreciable capacity and without direct connection to the apparatus serves as a lead, the question arises why even the salt solution outside the sheath, or the sheath itself, does not so serve, all along the nerve. The probability is that it does. So long, however, as a well-grounded electrode makes contact ahead of the travelling wave, the effect is shunted out, leaving the rising phase as recorded undistorted. The case is that where the more proximal lead (fig. 5b) has the lower resistance. When the action wave reaches the region between leads, there is undoubtedly a lead back along the nerve to the electrode as long as the excitation persists. The result is a slower apparent return of potential as recorded than actually takes place in the axons. When the nerve sheath at any point between leads is in

contact with a conductor, even with a drop of salt solution, or when the sheath is opened, the transverse resistance between the nerve fibers and the outside fluid is locally reduced, and a relatively low resistance path for current is established back to the ground electrode. The result is a localized second lead and a separate second wave following the first, instead of a mere prolongation of the first. It is obvious that the nearer the lead is to the killed end, the less will be the distortion of its falling phase. This distance is limited by other factors, however for instance the spread of injury from the killed area, which may extend at least as far as 1 cm.

From the above it may be seen that the potential as recorded from an electrode of appreciable width, is really recorded as it *leaves* the electrode, not as it reaches it; and measurements of distance should be made to the edge proximal to the other lead to the recording apparatus, not toward the stimulator.

f. Effect of nerve branches between leads. The same sort of distortion occurs when a nerve branch leaves the trunk at the region between leads. Having recorded a double action wave, as the wave passes an electrode and again as it passes the branch, the time of the second wave is not altered by laying the branch along the nerve in either direction, hanging it free, nor even by placing its cut end on the ground electrode. In other words, the effective lead is from the axons in the main trunk at the point where the branch leaves it, through the hole in the sheath where the branch emerges, the branch acting as an inert conductor between inside and outside of sheath. The position of this contact is not altered by cutting the branch shorter, though the resistance to the ground, and thus the amplitude of the second wave, may be altered. As many as four distinct waves, three of them due to branches, can be led from the bullfrog sciatic.

g. Double lead from two nerve components. If instead of cutting the branch, its killed end be extended to the grid lead, a single wave results, the two parts of the nerve merely recording in parallel. When, however, an attempt is made to record the wave in each part of the nerve separately, complications may result. For instance by putting tibial and peroneal, or motor and sensory roots of the sciatic, to separate grid leads with a common ground on the united nerve, the action potentials may be recorded separately or together by manipulating a grid switch. But no matter where the ground lead is along the nerve, at the junction of its two parts each may serve as a lead to the action wave of the other. Let us consider, say, the lead from the end of the peroneal and side of the sciatic, the end of the tibial being put to a separate electrode with an open switch. The axons of the peroneal now record the main wave as it passes the ground electrode on the nerve, as a monophasic wave; the

action wave in the tibial fibers however records at the ground electrode and at the junction with the peroneal, through the latter as an inert conductor to the grid, giving a diphasic wave from two points on live nerve. This diphasic effect overpowers any effect of the tibial as a branch between leads, and the summed effect is a slightly diphasic wave. Whether the branch causes a second wave or a trough thus depends on which predominates, the action wave from the axons of the main trunk through a leak, back to the ground lead, or the action wave from the axons of the branch, through the nerve forward to the grid lead.

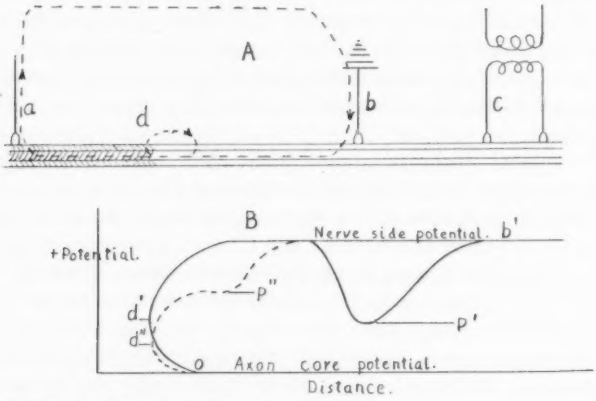

Fig. 6. Diphasic effects from monophasic leads.

A. *C*, cathode of stimulus; *b*, ground lead; *a*, grid lead from killed nerve to recording apparatus; *d*, killed region of nerve, and connective tissue sheath. Dash lines indicate current flow, with branch through apparatus.

B. Diagram of demarcation potential and action potential gradients near the killed region. *b'*, demarcation potential of live nerve, as registered on outside of sheath. *o*, theoretical potential of inside of nerve axons, *b' d' o*, potential gradient from outside of sheath to inside of live nerve through killed end. The lead *a* (in 6A), is effectively from a point in this gradient, *d'* not from *o*. Any change in the level of *b'* will change the whole gradient proportionally. The action wave when it reaches the vicinity of the killed end lowers the potential *b'*, and is thus recorded as a shift of the potential of *a* from *d'* to *d''*. *p'* represents the amplitude of a normal action wave first recorded at *b*, p'' that of the subnormal wave in injured tissue changing the gradient *b' d' o* to *p'' d'' o*, a part of which change *d' d''*, is recorded when the wave reaches the killed end as an action wave opposite in sign to that at *b*, because it makes *a* negative to *b*.

h. Effect of distance from killed end to lead. Another diphasic artifact may be produced if the ground electrode is at a considerable distance from the point at which the nerve is killed. A nerve is mounted in the usual way, and killed at the grid lead end (fig. 6-A). The ground lead *b* is mounted in a sliding holder to be moved along the nerve. Close to

the killed point d the action wave is strictly monophasic. As the distance between electrodes is increased, the wave becomes distinctly and progressively diphasic. If now we make the distance between leads, say, 2 cm., and observe a slight diphasicity, we can abolish this completely by killing the nerve with a hot rod at the half-way point, 1 cm. from each. Moving the ground electrode to 4 cm. restores the diphasicity, killing at 2 cm. or 3 cm. from the grid lead reduces or abolishes the effect. This can be repeated until the nerve is used up.

The effect may be accounted for by an analysis of the path of current from the excited region of the nerve to the two electrodes (fig. 6, B). We may assume that the main axon current flow from excited to inactive (or less active) nerve is inside the connective tissue sheath. A branch of this current however flows through the sheath, and thence, in part to the ground electrode, and in part along the nerve, leaking back through the sheath gradually to the axons. At the killed region, the demarkation potential gradient will fall, across the sheath, somewhat as in figure 6 B, the effective or average lead to a being at the potential of some point on this gradient, d'. As the wave crest reaches the killed region, there will be a change in the potential gradient from inside to outside of the sheath, and the effective lead connected by dead nerve to the grid will be somewhere on this changing gradient, at a potential more negative with respect to the unexcited side than before d''. If this killed area is far enough from the ground lead so that the region of the latter has recovered (positive) while the region near the killed end is still negative, the effect is to leave the grid negative $(d'-d'')$ until the excitation subsides in this region. If the ground electrode is close to the killed region, the two phases will nearly superimpose, and the action wave be merely shortened slightly in consequence, and reduced in amplitude. Since the amplitude near a killed area falls off gradually due to a spread of the injury, one cannot tell how much of the reduction may be due to this summation.

While the above account is based largely on inference, the change in diphasicity with distance of the ground lead from the killed region is what would be expected on these assumptions. Since the lead from the "dead" end is on a potential gradient from live to dead nerve, and thus affected by the action potential, if the two leads are close together the two phases will tend to sum algebraically and the one is more effective than the other. With greater distance, the two effects will separate out, and the second, even though slight, will show as a reversal of the first. According to the above the only strictly monophasic lead possible is at the junction between live and dead tissue, and at this point the excitation is so depressed as to be insignificant. The customary lead is an empirical compromise, and appears monophasic because it masks its own

error. The error, however, is generally slight, since the potential of the wave near the killed area is low ($b'p''$).

i. Death of part of fibers. A further effect can occasionally be demonstrated at a killed region. If the nerve dries slightly, or if for some other reason part of the fibers die back further toward the ground electrode then the rest, the monophasic wave may change rapidly during one series of observations to a slightly diphasic wave. In this case the death of fibers is *shortening* the distance between killed region and ground lead, and by analogy to the experience just previously described we should expect the opposite effect. The distances involved however preclude the former type of response, and the analogy is probably closer with the case of the diphasic lead at the crotch of a divided nerve. (See above, section *g*.) The dead fibers here apparently make a diphasic lead to the grid, from the live side of the fibers that have not died back so far. The next stage of the phenomenon is a marked decrease in amplitude of the wave, which is again full size at a distance slightly further from the killed area.

j. Resistance and capacity in leads. Beside the above factors, involving the structure and arrangement of the nerve, certain manipulations of the leading-off circuit cause characteristic distortion of the record. High resistance in series with the ground lead causes flickering of the record, and curvature of the base line, due to distortions picked up by the extremely low capacity grid lead from the rest of the apparatus. High grid resistance causes the same effect, together with a lowering of amplitude of a fast transient. A fairly satisfactory record can be obtained up to 150 or 200,000ω. With more apparatus capacity than we have in the amplifier grid input, resistance would have a correspondingly greater effect. Shunt resistance between grid and ground (in parallel with the nerve), acts theoretically only to cut down amplitude, but in practice leads to a reduction in the apparent duration of the wave. This is because the start and end of the wave approach the base line asymptotically, and reducing the amplitude reduces proportionally all parts of the potential gradient. If now we can detect a potential change only of a certain value, this value will be attained later on the rise, earlier on the fall of potential if the wave is reduced uniformly. In precise, comparative time measurements therefore all waves should be shunted to the same recorded amplitude. If we shunt a normal action wave to one-half amplitude it apparently shortens 0.000,05 second in time to maximum, 0.000,12 second in total duration. In fact the shunting of the nerve potential by the interstitial and connective tissue of the nerve itself must cause a similar error, and it has long been recognized that the recorded amplitude of potential is less than the actual. Removing the connective tissue sheath cuts down this error, and the high resistance of our apparatus (grid of amplifier), virtually eliminates its circuit as a nerve shunt.

For obvious reasons, shunting the nerve leads with capacity from grid to ground causes serious distortion, as does a resistance shunt (grid to ground) between a condenser which is in series with the grid, and the grid, an arrangement which is often useful in polarization experiments. In the latter case a lowering of amplitude, slowing of crest of the wave, and an apparent diphasicity results. Shunts in this case must be connected from between condenser and nerve, to ground.

DISCUSSION. Analysis of the above conditions not only prescribes the arrangements necessary to obtain a desired experimental result with a minimum of error, but also contributes to a knowledge of the behavior and constitution of nerves. One significant factor in nerve has been conspicuously ignored; namely, the presence of a connective tissue sheath. This acts as a longitudinal shunt, as has been recognized, but also as a transverse resistance to a stimulating current or to a led off action current. Moreover this sheath is a polarizable structure, and its polarizability undoubtedly affects not only the current density but also the time rate of rise of an applied stimulus. For this reason if its polarizability be markedly different from that of the axons, this should affect the apparent irritability of the nerve.

Further, our experiments tend to show that the sheath polarization is a large component in the "escape," and analogously, must produce distortion of the nerve's own potential as recorded. It, together with the high longitudinal resistance of nerve, tends to mask any change that may occur in the polarizability or permeability of nerve with excitation. It also tends to make the effective contact of an electrode with the nerve, however narrow, broader than the actual electrode surface.

Finally it must profoundly affect electrotonus, as measured, in the same manner as it affects the escape. If we consider a current flowing between two points of a nerve (either applied or excitation potential), when this current traverses the sheath it will polarize it, quite as it polarizes the axon surface. As a result of the counter E.M.F. established, the current will then spread to adjacent regions, and the polarization thus spread along the nerve. This will mask the true polarization phenomenon in the axons, which is presumably a similar process.

Thus most of the artifacts in the nerve records discussed above reduce to effects of the presence of, or alterations in the condition of, a structure which is not nerve at all. We cannot avoid the suspicion that much of the work on nerve resistance and permeability, electrotonus and polarization, liminal gradients, etc., needs reinterpretation in the light of the possible effects of a highly polarizable resistance being interposed between the nerve axon and the electrode. Further since polarization of the sheath and polarization of the nerve axons are presumably similar processes (differing in time relationships in general), knowledge of polariza-

tion of the sheath contributes to the knowledge of an essential nerve process. The axon must polarize, and whether this is the essential stimulus to nerve or not, polarization in both these structures must affect the time-strength relationships which vary so characteristically from nerve to nerve.

SUMMARY

Distortions of the record of the action potential in nerve can be traced, in part, to the physical characteristics of the recording and stimulating apparatus, but certain of them are due to the polarization and to the insulating properties of the nerve's connective tissue sheath.

a. The "shock escape" consists of three factors at least; 1, a primary escape into the amplifier input through the air; 2, a secondary escape down the nerve from the stimulating electrode through a circuit inside and outside the myelin and connective tissue sheaths, acting as core conductors, and 3, a spread of nerve or sheath polarization that outlasts the shock itself, similar to electrotonus.

b. Oscillating shocks may stimulate because the oscillations decrement, their algebraic sum acting as a current in one direction.

c. There is a transitory escape from make and break of a galvanic current stimulus, in addition to the constant escape during the current flow, which may be balanced out in a bridge circuit.

d. An electrode on the nerve not directly connected to ground, but with a capacity large compared to the amplifier grid, may serve as a ground lead.

e. If two electrodes are connected to ground, in parallel, from the nerve, the wave recorded may be reduplicated, the amplitude depending on the resistances in the ground leads. As the last electrode to ground, toward the grid lead, tends to shunt all the others, so the edge of any electrode toward the grid tends to shunt the rest of this electrode's contact with the nerve, and the excitation tends to be recorded where it *leaves* the electrode.

f. A cut branch *between the leads* to the recording mechanism may give rise to a second wave in the record as the excitation passes it, due to a leak from the main trunk through the sheath where the branch is cut and back along the outside of the sheath to the grounded electrode. An idle electrode on the intact sheath, a drop of salt solution, or a bit of wet undissected tissue may do the same, due to local reduction of resistance. If the terminus of the sheath at the division of the nerve into its spinal roots lies between the leads, a very large second wave results.

g. When the junction of the roots, or of the dichotomous division of the sciatic, lies between the electrodes, and the recording mechanism is connected with one branch only, the ground electrode on the undivided

nerve and the branch connected to the grid serve as two leads to the live side of the other branch, resulting in a diphasic action wave superimposed upon a monophasic one, the whole being slightly diphasic.

h. A nerve which is strictly dead at its end under the electrode may still give a diphasic wave, if the other lead is at a considerable distance, (two centimeters or more), from the edge of the killed area. This is because the lead from killed nerve changes in potential somewhat, as the excitation approaches the killed region. If the distance between leads is sufficiently great, the edge of the killed area may remain actively negative after the potential under the first electrode has subsided sufficiently to allow negativity at the killed end to show itself.

These experiments demonstrating the effectiveness of the nerve's connective tissue sheath as a resistance, which is also polarizable, suggest that many of the properties of nerve, as usually measured, may be in part the properties of this non-nervous structure.

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THE REGULATION OF RESPIRATION

IV. Tissue Acidity, Blood Acidity and Pulmonary Ventilation. A Study of the Effects of Semipermeability of Membranes and the Buffering Action of Tissues with the Continuous Method of Recording Changes in Acidity[1]

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Several years ago experiments by one of us pointed to a lack of correspondence between blood acidity and pulmonary ventilation, which seemed to contradict the generally accepted view that the acidity of the arterial blood controlled respiration. These experiments (1) led to the statement that "The conception that the hydrogen ion concentration of the arterial blood regulates respiration is analyzed and found wanting;" that "Parallelism between pulmonary ventilation and arterial pH is an accidental occurrence prevailing only under special conditions."

Considering respiratory control as a problem in transportation—the blood carrying acid to and from the cells and the metabolism of the cells determining the kind and amount of acid leaving and entering the cells—there was no reason for assuming a constant relation between blood and tissue acidity. It was pointed out that the more acid arterial blood may be the more efficient in carrying acid away from the tissues, that blood acidity can at best be only an indirect criterion of tissue acidity, that blood acidity and tissue acidity may vary in opposite directions as well as in the same direction. An effort to correlate the net effect of innumerable factors involved in changes of blood and tissue acidity under a variety of conditions pointed to a high coincidence of a direct relation of pulmonary ventilation to the acidity of the respiratory center itself. This led to the advancement of a working hypothesis connecting tissue acidity rather than blood acidity with the behavior of the respiratory center.

As to the lack of correspondence between pulmonary ventilation and blood acidity—that must be accepted as a fact. The graphic record of changes in acidity of the circulating blood leaves no room for doubt (2)

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

Results with the manganese dioxide electrode in hemorrhage and subsequent injection, mechanical asphyxia during the administration of room air and pure oxygen, the administration of sodium bicarbonate, ammonium chloride, adrenalin, nitrogen, etc., show the lack of correspondence between blood acidity and pulmonary ventilation. An inverse relation between blood acidity and pulmonary ventilation is by far the more common.

On the other hand a causal relation between tissue acidity and pulmonary ventilation should be considered as theory. And to avoid a common mistake of accepting theory as fact it is well to recall that the problem of relating functional activity of nervous structure such as the respiratory mechanism to fundamental processes still remains to be done (3).

One need only consider the effects of anesthetics and various salts on the respiratory center to realize that more than one factor plays a part in the control of respiration. We say that anesthetics depress the excitability of the respiratory center. During anesthesia there is a lack of oxygen, excess of carbon dioxide and hydrogen ions; nevertheless, respiration is lower than normal. But on the other hand, aside from the depression of activity, the center seems to follow the same general laws of response to respiratory stimuli.

The limited space prevents a discussion of fundamental problems involved in the automatic and reflex activity of nerve tissue. It should be recalled that the regulation of respiration in its ultimate analysis may be found to be an electrical phenomenon occurring with the aid of a surface membrane and change in composition of the fluids on both sides of that membrane. Whether acid is the only reagent which can produce the necessary electrical disturbance involved in the nerve impulse is highly improbable; on the other hand, it may be the agent most employed in the body.

The purpose of this research is to determine how closely theory is related to fact with respect to the effects of carbon dioxide, sodium carbonate and sodium bicarbonate. It represents an effort to learn, with various methods of approach, the directional changes in tissue acidity with synchronous changes in blood acidity; to follow the effects of permeability of cell membranes on tissue acidity and to ascertain the buffering action of tissues.

METHOD. The experiments were performed on dogs anesthetized with hypodermic injection of 10 mgm. morphine sulphate per kgm. body weight followed by rectal injection of 0.8 gram urethane per kgm. body weight. These injections usually maintain the animal in good anesthesia without marked depression of either respiration or circulation. Occasionally subsequent injections of urethane are necessary to insure greater regularity of breathing. Synchronous records of the acidity of the circulating arterial and venous blood were made with the manganese dioxide electrode as described in the preceding paper (4). The electrode vessels were placed in the course of the carotid artery and external jugular vein. In some experiments changes in acidity of the cerebro-spinal fluid were also recorded with the electrode vessel shown in figure 1. The original vessel consists of

a small glass T-tube, the vertical tube serving to hold the manganese dioxide electrode, and the right angle tube, filled with saline solution, serving to close the potentiometer circuit. The dog is placed on its side and the occipito-allantoid membrane and dura over the fourth ventricle are exposed by reflecting the neck muscles with thermocautery. A pursestring suture is made over the center of the membrane and the lower end of the electrode vessel, which is provided with a ring of DeKhotinsky cement, is passed through a small incision into the fourth ventricle. The pursestring suture is then secured and about 50 grams traction applied to the electrode vessel with string and pulley at right angles to the membrane. This prevents injury and hemorrhage by keeping the point of the electrode and the electrode vessel away from the floor of the fourth ventricle despite gross movements of the head of the animal during heavy ventilation. The greatest precaution should be employed to prevent hemorrhage as the presence of blood, due to the effects of oxidation and reduction, influences the change in acidity of the cerebro-spinal fluid. For purposes of checking the validity of the data provided by the manganese dioxide electrode an additional tube was cemented with DeKhotinsky cement to the electrode vessel to provide samples of cerebro-spinal fluid.[2] The pH of the samples was determined with the quinhydrone electrode, as in the preceding paper. Blood pressure was recorded with the mercury manometer and pulmonary ventilation with rebreathing tanks. The animal was connected by a single inspiratory and expiratory valve with three tanks of seventy liters capacity each. This arrangement allows a quick shift from one gaseous mixture to another. The tanks are provided with soda lime cartridges so that basal metabolism as well as pulmonary ventilation may be followed. The rate of oxygen consumption is indicated by the gradient of the respiratory record.

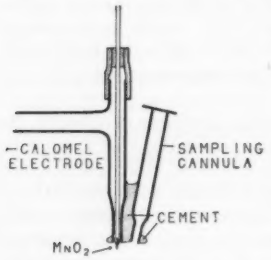

Fig. 1. Combination cerebro-spinal fluid electrode vessel and sampling tube. In most of the experiments the electrode vessel with the sampling tube detached was used.

RESULTS. Figure 2 shows the effects of administration of carbon dioxide directly into the trachea while the animal was connected with a rebreathing

[2] The cerebro-spinal fluid electrode and sampling vessel has been markedly improved by constructing it from a block of transparent bakelite. The ring of de-Khotinsky cement which has pulled off the glass vessel during the course of several experiments is replaced by a 30° angle-shaped groove. On tightening the purse string suture the membrane fits snuggly in the groove. The sampling tube is of finer bore just permitting the passage of the syringe needle. The upper end of the sampling tube opens on an offset which is covered with rubber dam.

tank filled with room air. The upward bend of the respiratory record is the result of the administration of carbon dioxide and the sudden downward bend is due to the absorption of the gas at the end of administration. In all subsequent records gaseous mixtures are administered as described above.

The effects of administration of carbon dioxide shown in figure 2 are increased acidity of the arterial and venous blood accompanied by increased pulmonary ventilation. These effects are followed during recovery by decreased acidity of the arterial and venous blood and decreased pulmonary ventilation. The sequence of events is designated. The electrode in the carotid blood indicates increased acidity first and is followed in about ten seconds by the same indications in the jugular blood. Careful measurement of the respiratory record shows that the arterial electrode responds to increased carbon dioxide before the respiratory center, and, similarly, that the venous electrode responds to increased carbon dioxide after the respiratory center. That is, the respiratory center which is midway between the two electrodes responds when the carbonated blood arrives at the center midway between points 1 and 2 on the figure.

Figure 2 represents the type of data which was largely responsible for the view that acidity of the blood controls pulmonary ventilation. The stimulating effects of the carbon dioxide and the depressing effects of sodium carbonate represent two of the few instances of parallelism between arterial acidity and pulmonary ventilation, and it is shown that in these two instances the changes in blood acidity reflect similar changes in tissue acidity. Accepting the view that tissue acidity (in this case acidity of the respiratory center) is the dominant chemical factor controlling respiration, an explanation of the effects of carbon dioxide becomes apparent (fig. 2). In following the shape and magnitude of the arterial and venous acidity curves one is impressed with the greater change and the greater abruptness of the change in acidity in the arterial blood both during the administration of carbon dioxide and during recovery. It is possible that the greater magnitude of the acidity change on the arterial side may be explained in part by the effects of reduction of oxyhemoglobin on the passage of the blood from the arterial to the venous side of the tissues but the change in the gradients of the acidity curve resulting from the flow of blood through the tissues seems explainable primarily by the passage of carbon dioxide between the blood and tissue. The record indicates that it requires an appreciable amount of time for the tissues to saturate with carbon dioxide on administration and to desaturate during recovery. (Note the very late recovery on the venous side.) In that event the venous blood must contain less carbon dioxide than the arterial blood during the administration of carbon dioxide and more during recovery; that is, the tissues have acted as buffers to the blood and in the process have temporarily become more acid themselves.

An exaggerated example of similar conditions is shown in figure 3, illustrating the effects of a prolonged administration of a twenty per cent mixture of carbon dioxide in room air. The arterial change in acidity is again abrupt, and recalling the appreciable time required for the manganese

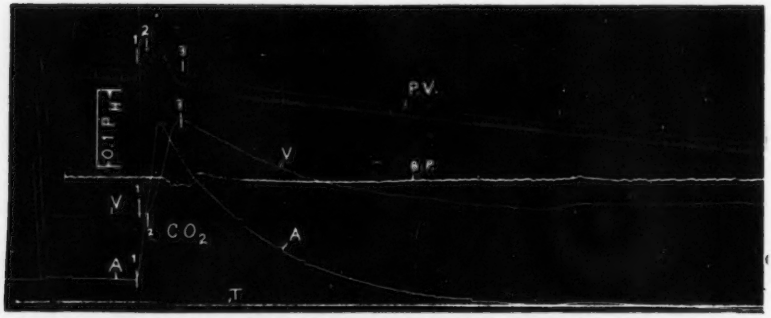

Fig. 2. Effects of administration of carbon dioxide directly into trachea of dog connected with rebreathing tank filled with room air. *P.V.*, pulmonary ventilation; *B.P.*, mean blood pressure; *V*, acidity curve of venous blood (external jugular vein); *A* acidity curve of carotid blood; *T*, time in seconds. Duration of record 15 minutes. The extent of the deflection per 0.1 pH is indicated by the vertical scale. Venous pH change, 0.12.

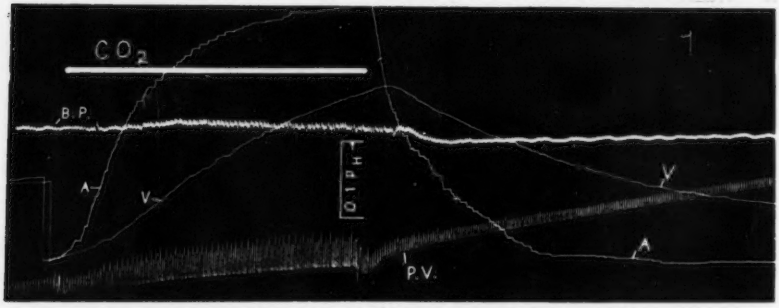

Fig. 3. Effects of administration of a 20 per cent mixture of carbon dioxide in room air for a period of 6 minutes. CO_2, duration of administration of carbon dioxide. Total duration of record 15 minutes. Arterial pH change, 0.32. Venous pH change, 0.20. *B.P.*, blood pressure; *A*, carotid blood acidity curve; *V*, jugular blood acidity curve; *P.V.*, pulmonary ventilation. The extent of the deflection per 0.1 pH is indicated by the vertical scale.

dioxide electrode to come into equilibrium with the blood itself, it is obvious that the arterial blood comes into equilibrium with the change in alveolar gases more completely than the record suggests. On the whole all our records indicate that equilibrium is rapidly established between the blood and the alveolar gases and only slowly between the blood and the tissues,

indicating the great capacity of the tissues to buffer. In figure 3 the venous record again shows a relatively gentle gradient on the administration of carbon dioxide, indicating a progressive saturation of the tissues with the gas. This in turn is followed by a gentle recovery gradient which indicates a progressive desaturation of the tissues. Recovery of the venous blood to the original acid level is decidedly incomplete within the limits of the published record.

Not infrequently the acidity of the arterial blood may fall below the normal level during recovery. An explanation of this and other details will be left for later papers.

Figures 4, 6, 8 and 11 show the effects of intravenous injection of sodium carbonate. The effects are increased alkalinity of the arterial and venous blood, decreased respiration and a fall in blood pressure followed by decreased alkalinity of the arterial and venous blood, increased respiration and increased blood pressure. Making the same comparison as in the

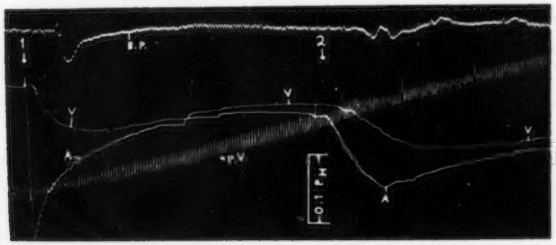

Fig. 4. A comparison of the buffering action of tissues with a rapid and slow intravenous injection of 8.0 cc. 0.5 M Na_2CO_3. Weight of dog, 9.5 kgm. *B.P.*, mean blood pressure; *V.*, jugular blood acidity curve; *A*, carotid blood acidity curve; *P.V.*, pulmonary ventilation. Duration of record, 12 minutes. First arterial pH change, 0.20. First venous pH change, 0.07. Second arterial pH change, 0.1. Second venous pH change, 0.06.

records showing the effects of carbon dioxide it is noted that the arterial changes precede the respiratory response which in turn precedes the venous change. The arterial changes in pH are likewise greater and more abrupt than the changes in the venous blood. The records indicate that the tissues have acted as buffers to the alkalinized blood as well as to the acidified blood. Another such example is found in figure 5 which shows the effects of the intravenous injection of sodium hydroxide.

In contrast to the experiments on the administration of carbon dioxide the smaller change in pH in the venous blood cannot be attributed to the alkaline effect of the reduction of oxyhemoblogin, for in this instance the venous blood turns less alkaline than the arterial. The smaller increase in alkalinity of the venous blood may, therefore, be attributed in the main to

the buffering action of the tissues. Conceivably this buffering may occur as a result of the passage of base from the blood into the tissues and of the passage of carbon dioxide from the tissues into the blood. Either process would turn the blood more acid and the tissues more alkaline and according to theory account for the changes in pulmonary ventilation and blood pressure. The relative contribution of each of these two processes to the changes in tissue acidity and the behavior of respiration and circulation is perhaps suggested in a qualitative way by some of the later experiments to be described in this paper.

The next few records are only a further indication of the extent and significance of the buffering action of the tissues. Varying degrees of buffering by the tissues can be demonstrated by varying the diffusion gradient of the radicles involved in acid-base equilibrium between the blood and tissue. A rapid injection of sodium carbonate will momentarily lower the carbon dioxide tension of the arterial blood more than a slow injection, and, similarly, it will raise the concentration of base in a circulating block of blood more than a slow injection. In figure 4 the buffering effect of the tissues with a rapid injection is enormously greater than with a slower injection. The greater fall in blood pressure and diminution of respiration with the rapid injection, according to theory, would be explained by a greater increase in tissue alkalinity.

The buffering action of the tissues may be varied in a somewhat similar way as illustrated in figure 6. Reasoning that a slower flow of blood should permit the establishment of a more perfect equilibrium between blood and tissue on the injection of sodium carbonate and thereby increase the buffering action of the tissues, equal amounts of sodium carbonate were injected at equal rates before and after clamping both carotid arteries. The upper record shows the arterial and venous changes in alkalinity resulting from an injection with the normal volume flow of blood. The lower record shows the pH changes in the venous blood produced by injection when both carotids were clamped. (As the arterial electrode is in the carotid artery the arterial record is missing.) In the lower record the pH change in the venous blood comes decidedly later and is appreciably smaller than in the upper record. The later appearance indicates the slowing of the blood stream and the smaller change indicates the greater buffering by the tissues.

The effect of carbon dioxide on arterial and venous pH shown in figure 7 before and after hemorrhage is another demonstration of the variability of the buffering action of the tissues dependent upon the volume-flow of blood. In such experiments the effects of volume-flow of blood on changes in acidity of the arterial blood within the lungs are shown as well. Note the steepness of the arterial curve during administration of carbon dioxide in the lower record following hemorrhage as compared with the upper

record preceding hemorrhage. It seems only reasonable to expect a more complete saturation of the blood with carbon dioxide if greater opportunity

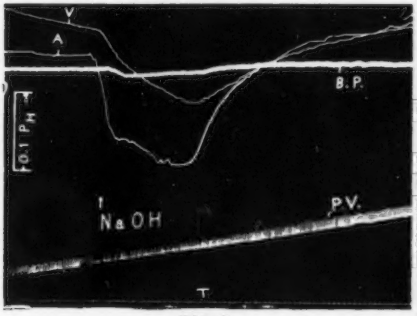

Fig. 5

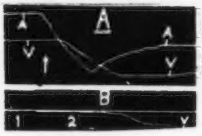

Fig. 6

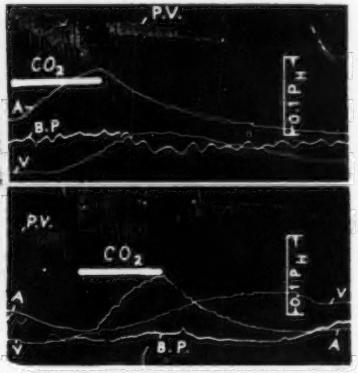

Fig. 7

Fig. 5. Intravenous injection of 13.5 cc. 0.4 M NaOH. Weight of dog, 8.5 kgm. *V.*, jugular blood acidity curve; *A*, carotid blood acidity curve; *P.V.*, pulmonary ventilation; *T*, time in six second intervals. Total time, 10 minutes.

Fig. 6. Effect of volume-flow of blood on the buffering of the blood by the tissues. Records A and C show the changes in alkalinity of the carotid and jugular blood on the intravenous injection of sodium carbonate with both carotids open. Record B is a corresponding curve showing the effect of occlusion of both carotids on the alkalinity change in the venous blood. *A*, carotid blood acidity curve; *V*, jugular blood acidity curve. The arrows indicate the moment of injection.

Fig. 7. Effect of volume-flow of blood on the buffering action of the tissues on the administration of carbon dioxide. The upper record shows the effects of administration of carbon dioxide before hemorrhage and the lower record after hemorrhage. *P.V.*, pulmonary ventilation; CO_2—duration of administration of carbon dioxide; *A*, carotid blood acidity curve; *B.P.*, mean blood pressure; *V.*, jugular blood acidity curve.

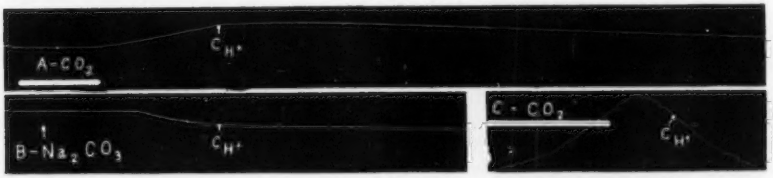

Fig. 8. Changes in acidity in the tissues produced by the administration of carbon dioxide and sodium carbonate. In records A and B the manganese dioxide electrode was placed between the fibers of the sartorius muscle. In record C the electrode was placed between the cerebral convolutions.

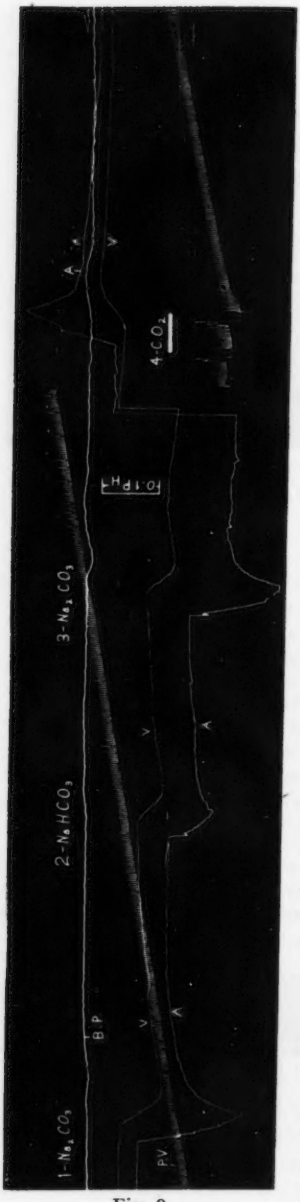

Fig. 9

is offered for absorption. And, similarly, it seems as reasonable that the tissues will have greater opportunity to absorb this carbon dioxide when the blood flows more slowly through the tissues. Hemorrhage then should sharpen the arterial curve and blunt the venous curve (see fig. 7).

Granting that the tissues are functioning in this buffer capacity the effects of changes within the blood stream exerted outside the blood stream should be demonstrable. Figure 8 shows changes in acidity recorded with the manganese dioxide electrode placed between the fibers of the sartorius muscle on the administration of carbon dioxide (record A) and on the injection of sodium carbonate (record B). The changes in pH are smaller and occur more slowly than the changes in the blood. In record C, however, where the electrode was placed between the cerebral convolutions the change is large and rapid.

The freedom of movement of carbon dioxide through cell membranes and its rapid rate of diffusion in solution suggests the simplest explanation of buffering action of the tissues, but a consideration of the movement of base and the restraints offered to that movement is equally essential. Whether the restraint offered to the movement of base is of the nature of a slow diffusion or of the nature of a barrier in the form of impermeability of membranes, the local effect is momentarily the same. The stratification of two solutions of the same pH $H_2CO_3/NaHCO_3 = 1/20$ and $H_2CO_3/NaHCO_3 = 3/60$, results in a change

Fig. 9. Comparative buffering effects of the tissues on the blood following the administration of sodium carbonate, sodium bicarbonate, and carbon dioxide. Greater similarity in the arterial and venous acidity curves are frequently obtainable on the injection of sodium bicarbonate. *B.P.*, blood pressure; *V*, jugular blood acidity curve; *A*, carotid blood acidity curve; CO_2, duration of administration of carbon dioxide.

of pH at the interface comparable to the change which would occur if the solution were separated by a semipermeable membrane (5). The significance of restraints to movement of base, whatever the nature of the restraint may be, is indicated in figure 9—a continuous record showing the effects of injection of sodium carbonate, sodium bicarbonate and carbon dioxide. The typical buffering effects of the tissues on the injection of sodium carbonate and on the administration of carbon dioxide are present. But on the injection of sodium bicarbonate the buffering effects of the tissues are absent. There is a great similarity in magnitude and contour of the arterial and venous acidity curves which agree with the results anticipated. It will be recalled that one of us, on a previous occasion, suggested that the so-called "specific" action of sodium bicarbonate and carbon dioxide (Collip (6), Dale and Evans (7) and others) might be explained on an acid basis.

The argument presented at that time is so essential for an understanding of the present paper that it is given in part again (1).

We have recently (5) demonstrated a striking disturbance in the hydrogen ion equilibrium of a solution without the use of a membrane. We prepared a relatively weak and a relatively strong solution of sodium bicarbonate and with the aid of phenol red and carbon dioxide raised the hydrogen ion concentration of each to the same point. The two solutions were carefully stratified in a cylindrical separatory funnel. The denser solution being below, none of the constituents of the solutions can mix except by the process of diffusion. In a few moments a light colored acid ring appears at the interface. This ring gradually increases in width and simultaneously a dark alkaline ring develops below. The formation of colors widens and slowly ascends.

The explanation of the phenomena is, of course, obvious. A substance in solution tends to diffuse from a point of high concentration to a point of lower concentration. Sodium bicarbonate and carbon dioxide will, therefore, tend to diffuse upwards, but as a result of the higher rate of diffusion of carbon dioxide the disproportionate entrance of carbon dioxide in the upper solution increases the $\frac{H_2CO_3}{NaHCO_3}$ ratio, and, similarly, the disproportionate departure of carbon dioxide from the lower solution decreases the ratio with a consequent display of colors. An additional differential barrier, for example, the impermeability of the membrane to the sodium bicarbonate, should of course accentuate the exceedingly rapid process exemplified by the simple diffusion experiment. Undoubtedly, such hindrance occurs between the blood and the nerve cell.

Another possible explanation also occurred to us. It is known that when carbon dioxide is dissolved in water it is present in three different forms—dissolved CO_2, undissociated H_2CO_3 and dissociated H_2CO_3. Thus $CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H.CO_3'$. Addition of sodium bicarbonate pushes the reaction to the left increasing both the dissolved CO_2 and the undissociated H_2CO_3. This effect alone, neglecting the formation of carbon dioxide in the tissues, would tend to produce diffusion of carbon dioxide into the tissues; that is, the injection of sodium bicarbonate should theoretically produce acidosis along with alkalemia by virtue of the greater accumulation of carbon dioxide in the tissues.

But this conception neglects entirely the effects of the H· and HCO′ ions. We

know, for example, that the H· ion, within the blood at least, shifts with extreme rapidity between the cells and the plasma with every circuit of the blood. If so, there appears to be no reason why dissociated carbonic acid should not also diffuse which suggests another possible stimulating effect of sodium bicarbonate.

If we stratify a solution of hydrochloric acid with water, a definite number of chlorine ions will tend to leave the acid for the water. In so doing they will pull the hydrogen ions with them and turn the water acid. This process is, of course, accelerated by the further addition of chlorine ions in the form of hydrochloric acid to the acid solution. The addition of neutral sodium chloride is also effective in accelerating the diffusion of the acid. This addition increases the number of chlorine ions tending to leave the acid solution for the water, but inasmuch as the chlorine ions cannot leave by themselves, they will take either H or Na ions with them. The H ion being the more mobile is the more easily pulled from solution and in consequence acid diffuses more rapidly than before.

It is obvious that the conditions are somewhat different in the case of a weak acid, such as carbonic acid, and its salt, sodium bicarbonate, for the addition of the salt markedly reduces the hydrogen ion concentration of the solution. Yet in so doing it has not only increased the amount of freely diffusible undissociated CO_2 and H_2CO_3, but has increased the rate of diffusion of the H ions which have been decreased in number. So far as the acidity of the interior of the living cell placed in such a solution is concerned, the effectiveness of the remaining hydrogen ion is theoretically increased for presumably the relative impermeability of the cell membrane to the sodium ion will prevent an accelerated diffusion of the added bicarbonate. Theoretically, at least, the intravenous injection of sodium bicarbonate should exert a double effect, preventing the diffusion of carbon dioxide from the tissues into the blood, by increasing the concentration of H_2CO_3 and CO_2 in the blood and preventing the diffusion outwards from the cells of the HCO_3' ion to which the H. ion is attached.

Returning to the significance of figure 9 of this paper—if base is hampered in its movement from blood to tissue, the tissues would buffer mainly through a movement of carbon dioxide (consideration of the shift of chlorine and other anions is omitted). But, on the other hand, if the movement of carbon dioxide from tissue to blood is also hampered buffering is prevented on this score too. In that event the blood may pass through the tissues with approximately the same acidity curve with which it entered the tissues, and such is the common finding in a large number of experiments.

By placing an electrode in the fourth ventricle and recording the changes in acidity of the cerebro-spinal fluid perhaps a better opportunity is offered for studying the restraining effects on the movement of base between blood and tissue. In such experiments a living membrane is interposed between the blood and the electrode in the tissue fluid. Such experiments have value in that they may indicate the directional changes in tissue acidity associated with changes in blood acidity.[3]

[3] An occasional difficulty to the continuous method of recording changes in acidity of the cerebro-spinal fluid is the rapid E.M.F. drift and dissolution of the electrode. The manganese dioxide plating may completely dissolve within an hour though

Figure 10 illustrates the effects of administration of carbon dioxide: increased acidity of the arterial and venous blood and cerebro-spinal fluid,

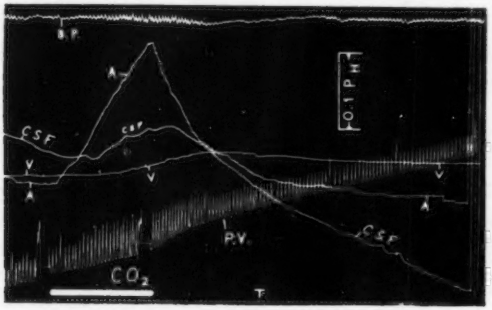

Fig. 10. Effects of administration of carbon dioxide on the acidity of the arterial and venous blood and the cerebro-spinal fluid. There is a very extensive drift in E.M.F. in the cerebro-spinal fluid electrode which must be corrected for as described in the preceding paper. *B.P.*, mean blood presure; *A*, carotid blood acidity curve; *C.S.F.*, cerebro-spinal fluid acidity curve; *V.*, jugular blood acidity curve, *P.V.*, pulmonary ventilation; CO_2, duration of administration of carbon dioxide.

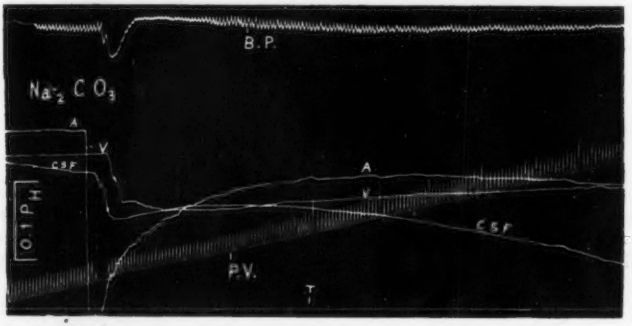

Fig. 11. Effects of intravenous injection of sodium carbonate on the acidity of the arterial and venous blood and cerebro-spinal fluid. Note the drift in the cerebro-spinal fluid acidity record. Weight of animal 10.5 kgm. *B.P.*, mean blood pressure; Na_2CO_3 intravenous injection of 25 cc. 0.5 M sodium carbonate; *A.*, arterial blood acidity curve; *V.*, jugular blood acidity curve; *C.S.F.*, cerebro-spinal fluid acidity curve; *P.V.*, pulmonary ventilation; *T.*, time in 6-second intervals.

usually the electrodes last several hours and give excellent records. A slight amount of protein from exceedingly small hemorrhage into the fourth ventricle seems to prolong the life of the electrode. Figure 12 shows a good behavior of the electrode and figure 10 a rapid drift. The rapid drift and disappearance of the manganese dioxide may be due to an abundance of reducing substances in the cerebro-spinal fluid. The protective action of blood is not so clear.

and increased pulmonary ventilation. The results are comparable to those shown in figure 8, where the electrode was placed between the cerebral convolutions.

Figure 11 shows the effects of intravenous injection of sodium carbonate: increased alkalinity of the arterial and venous blood and cerebro-spinal fluid. Accompanying these changes there is a decrease in respiration and a fall in blood pressure. Assuming for the moment that the behavior of cerebro-spinal fluid represents the changes in pH of the tissues, the explanation of the associated respiratory phenomena on an acid basis is supported.

In figure 12 the effects of intravenous injection of sodium bicarbonate are shown. There is an increased alkalinity of the arterial and venous blood, and increased acidity of the cerebro-spinal fluid. The alkalinity of the arterial and venous blood is long maintained and shows very little tendency

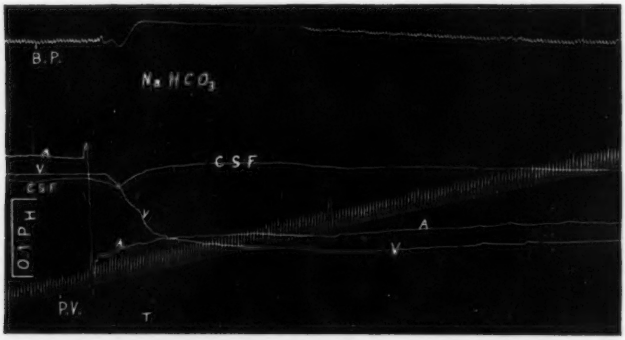

Fig. 12. Effects of intravenous injection of sodium bicarbonate on the acidity of the arterial and venous blood and cerebro-spinal fluid. *B.P.*, mean blood pressure; *A.*, carotid blood acidity curve; *V.*, jugular blood acidity curve; *C.S.F.*, cerebro-spinal fluid acidity curve; *P.V.*, pulmonary ventilation; *T*, time in 6-second intervals.

to recover to its original level, but despite this changed reaction the cerebro-spinal fluid acidity curve holds almost horizontal at its new acid level. At the end of ten minutes it is still distinctly more acid than normal. Associated with this increased acidity there is a prolonged increase in blood pressure and an increase in pulmonary ventilation outlasting the duration of the published record. The close parallelism between the circulatory and respiratory response and the acidity of the cerebro-spinal fluid cannot but support the previous suggestion of a causal relation between the two phenomena (3).

Theoretically, at least, the intravenous injection of sodium bicarbonate simply by increasing the concentration of CO_2, H_2CO_3, and HCO_3' ions in the blood prevents the diffusion outwards of the CO_2, H_2CO_3 and the HCO_3' to which the H. ion is attached. The acid formed in the tissues should, therefore, accumulate. If this is true it is a principle of utmost importance in the physiology of respiration.

The significance of such findings applied to tissue acidity is too great to omit a check on the acidity changes occurring within the cerebro-spinal fluid itself. Previous checks with the hydrogen and quinhydrone electrode have demonstrated the value of the data supplied by the manganese dioxide electrode in the circulating blood. Similar checks have been repeated on the cerebro-spinal fluid. By inserting a sampling tube

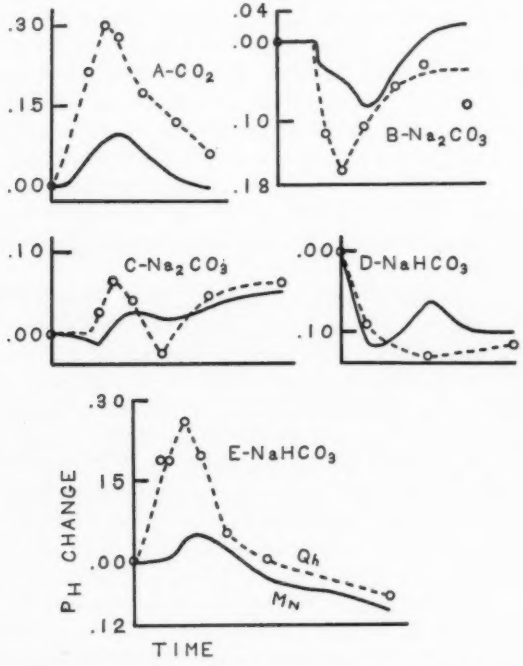

Fig. 13. Comparison of cerebro-spinal fluid acidity curves obtained with the continuous method (manganese dioxide electrode) with curves established with the discontinuous method (quinhydrone electrode). *A*., administration of carbon dioxide; *B* and *C*, intravenous injection of sodium carbonate; *D* and *E*, intravenous injection of sodium bicarbonate.

into the fourth ventricle, small samples (0.2 cc) of cerebro-spinal fluid were withdrawn and the pH value determined with the quinhydrone electrode. A comparison of the curves with the graphic records obtained with the manganese dioxide electrode is shown in figure 13: record A—the administration of carbon dioxide, records B and C—the injection of sodium carbonate, records D and E — the injection of sodium bicarbonate. The curves show directional agreement throughout with the exception of

one observation in record D, in which the manganese dioxide curve shows an extra acid swing. For some unknown reason this effect occasionally resulted from drawing a sample.

Quantitative agreement is not indicated in the figures but as no effort was made to determine the E. M. F. changes per 0.1 pH in cerebro-spinal fluid in in vitro experiments quantitative comparison is impossible. The chief concern at this stage of the problem was to establish whether or not the manganese dioxide electrode is a reliable indicator of directional changes in acidity in the cerebro-spinal fluid. Unfortunately we cannot be too certain of the validity of checks with the quinhydrone electrode for as was pointed out before the potential of both electrodes is affected by reducing substances, but inasmuch as it was necessary to remove very small samples of cerebro-spinal fluid the quinhydrone electrode was adhered to. As will appear below there were no indications of altered metabolism which might vary the amount of reducing substance. All that can be said at present is that the check with the quinhydrone electrode confirms the manganese dioxide electrode.

Accepting the results as valid, are we justified in accepting the proposed explanation of the acid effects of bicarbonate (1) (3) (5) based on the relative shift of acid and base determined by the rate of diffusion of and the impermeability of the membrane to the anions and cations of a bicarbonate buffer mixture? It is desirable to rule out other possible factors which might account for the increased acidity of the cerebro-spinal fluid such as circulatory disturbances and basal metabolism. It is hard to see how circulation of the blood could account for the increased acidity of the cerebro-spinal fluid, for Doctor Bronk and one of us have shown (unpublished work with the continuous thermophile method (8) of recording volume flow of blood) that the carotid flow of blood is enormously increased by the injection of bicarbonate. Neither are there indications that changes in basal metabolism (see records) could account for the acid changes in the cerebro-spinal fluid. In addition—unpublished experiments by Dr. McGinty and one of us, with the continuous method of recording changes in expired carbon dioxide and oxygen, show an increased elimination of carbon dioxide through the lungs on the administration of sodium bicarbonate. It is possible that sodium bicarbonate acts in the same way in the lungs and tissues in expelling acid from the blood. We are for the present forced back to the effects of sodium bicarbonate on the buffer mixtures in the blood and on the impermeability of membranes. Certainly figure 12 suggests a high degree of impermeability to base of the membranes determining the composition of the cerebro-spinal fluid. The prolonged maintenance of the increased acidity of the cerebro-spinal fluid despite the maintained alkalinity of the blood is strongly supporting evidence. On the other hand, if conditions existed for the passage of both

carbon dioxide and sodium bicarbonate into the cerebro-spinal fluid but in an acid ratio, for example, greater than $\frac{1}{20}$, the cerebro-spinal fluid would turn acid too. Such a movement of acid and base is not inconceivable. As a matter of fact it has been demonstrated in the stratification experiments without the participation of a membrane. The same condition might result in the cerebro-spinal fluid and would also be analogous to the conditions which appear to exist at the arterial electrode immediately following an injection of sodium bicarbonate (see fig. 12). There is an indication of initial increased acidity possibly resulting from the advance migration of acid followed by a reverse deflection possibly due to increased alkalinity resulting from the arrival of the more slowly moving sodium at the electrode. But the prolonged change in reaction of both the cerebro-spinal fluid and blood in figure 12, as contrasted with the momentary changes at the electrode, speak against this explanation.

Other evidence indicating the significance of impermeability is found in the effects of sodium carbonate. Not infrequently sodium carbonate exerts both alkaline and acid effects upon the cerebro-spinal fluid. An initial alkaline effect associated with decreased blood pressure and pulmonary ventilation is followed in several minutes by increased acidity and increased ventilation and blood pressure above the normal values. Provided the membranes involved possess a high degree of impermeability to base, the initial alkaline effect is explainable by the extraction of carbon dioxide from the tissues into the blood. But once the carbonate has changed to bicarbonate and free carbon dioxide again begins to accumulate, the back pressure produced by the increased bicarbonate should lead to further accumulation of acid in the tissues. The fact that carbonate exerts this acid effect after a lapse of three to five minutes, during which time base might conceivably enter the tissues very freely were the membranes permeable to base, suggests a high degree of impermeability to sodium.

The degree of impermeability, however, seems to vary. With very large injections of sodium bicarbonate correspondingly large increases in acidity of the cerebro-spinal fluid occur—sometimes as large as 0.2 to 0.3 pH—but after the initial increase in acidity a relatively rapid alkaline drift occurs. This drift might be explainable either by a parallel elimination of bicarbonate by the kidneys, which would decrease the back pressure of the sodium bicarbonate, or by an increased permeability of the cerebral membranes. We have shown a rapid elimination of alkali by the kidneys following the injection of sodium carbonate (9). On the other hand, an initial alkalinity of the cerebro-spinal fluid, following the administration of sodium bicarbonate, as shown in figure 14, suggests, in that particular experiment, a free movement of base through a freely permeable membrane. The fact that such results are obtained more frequently toward the close of an experiment or after severe abuse, such as the administration of low

oxygen or sodium cyanide, points to injury of the membranes. It is interesting to note that when sodium bicarbonate produces these alkaline changes in the cerebro-spinal fluid it likewise depresses respiration and blood pressure.

Occasionally the administration of sodium bicarbonate may have no effect whatever on pulmonary ventilation. Is this due to a passage of acid and base in a non-stimulating ratio? Obviously this phase of the subject requires further critical experiments for an ultimate understanding of the effects of sodium bicarbonate. Chemical analyses of the cerebro-spinal fluid are desirable. Some have been made but not to our entire satisfaction. The difficulties opposing clean cut observations though perhaps not insurmountable are considerable. Such results as have been obtained adapt themselves to the views so far expressed: for example, a

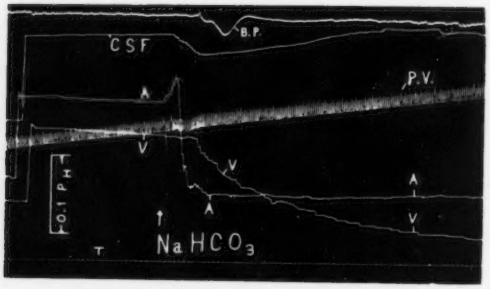

Fig. 14. Observation showing alkaline effect of the intravenous injection of sodium bicarbonate on the cerebro-spinal fluid. *B.P.*, mean blood pressure; *C.S.F.*, cerebro-spinal fluid acidity curve; *A.*, carotid blood acidity curve; *V.*, jugular blood acidity curve; *P.V.*, pulmonary ventilation.

decreased carbon dioxide content of the cerebro-spinal fluid following the administration of sodium carbonate has been found which indicates high degree of impermeability, and also increased carbon dioxide content during the alkaline drift following the increased acidity of the cerebro-spinal fluid elicited by injection of sodium bicarbonate which indicates abnormal permeability. We hope to have more complete chemical data in the future but for the present we are inclined to believe that the data at hand suggest that the behavior of the membrane plays an important part in explaining the effects of sodium bicarbonate.

Discussion. As the logical method of presentation was used there is little need for further discussion. There is one point, however, which calls for more attention, namely, to what extent are we justified in assuming that changes in acidity of the cerebro-spinal fluid reflect the changes in acidity of the respiratory center. There is no reason for believing that the acidity

of the cerebro-spinal fluid is an infallible index to the acidity of the respiratory center.

In evaluating the significance of the acidity of the cerebro-spinal fluid with respect to the acidity of the brain, it should be recognized that the cerebro-spinal fluid is subjected to several influences which may affect its acidity. Not only is it affected by the permeability of membranes, but by the metabolism of the nerve cells which determines the acid contribution of the brain to the fluid and the ventilating effect of the choroid plexus which determines the amount of acid brought to and taken away from the cerebro-spinal fluid.

In these experiments there are no obvious reasons for believing that altered metabolism plays a rôle in the interpretation of results. On the other hand the increased blood flow through the brain on the administration of carbon dioxide exerts a ventilating effect in bringing large amounts of acid to the brain and the increased flow of blood on the administration of sodium carbonate improves ventilation by increasing the transport of acid away from the cerebro-spinal fluid. But sodium bicarbonate also increases the flow of blood. What then is the ventilating effect in this instance? Obviously if increased flow of blood were the only effect of sodium bicarbonate the cerebro-spinal fluid should turn alkaline. In general, the extent of the ventilating effect of the choroid plexus is determined by the flow of blood. The directional effect is determined by the composition of the blood.

If this logic is correct there is reason for believing that in these particular experiments the changes in acidity of the cerebro-spinal fluid is a fair index to the changes in acidity of the respiratory center. But differences are not inconceivable. There is no reason for believing that the membranes involved in functional activity of the respiratory center are identical in behavior to the membranes responsible for the composition of the cerebro-spinal fluid. But on the whole—the agreement between pulmonary ventilation and acidity of the cerebro-spinal fluid is good.

SUMMARY

The chemical regulation of respiration was studied with the continuous method of recording changes in acidity in the circulating blood and body fluids.

The relation of respiration to the acidity of the respiratory center was analyzed by a study of the changes in acidity of the arterial and venous blood and the cerebro-spinal fluid.

Comparison of the arterial and venous blood acidity curves gave information on the passage of acid and base between blood and tissue. It indicated the extent of buffering by the tissues and the consequent changes of acidity in the tissues. Synchronous records of the acidity of the cerebro-spinal

fluid gave collateral evidence on the effects of semipermeability of membranes on acid-base equilibrium and tissue acidity.

The administration of carbon dioxide increased the acidity of the arterial and venous blood. The changes in acidity were greater and more abrupt on the arterial side of the tissues than on the venous side. This suggested a passage of carbon dioxide from the blood into the tissues during the administration of carbon dioxide and a passage from the tissues into the blood during recovery. The tissues accordingly acted as buffers to the blood and in the process became more acid. The increased acidity of the tissues was accompanied by increased pulmonary ventilation.

The intravenous injection of sodium carbonate increased the alkalinity of the arterial and venous blood. The changes in alkalinity were greater and more abrupt on the arterial side of the tissues than on the venous side. The hampered movement of base suggested a passage of carbon dioxide from the tissues into the blood on injection and retention of carbon dioxide by the tissues during recovery. The tissues accordingly acted as buffers to the blood and in the process became more alkaline. The increased alkalinity of the tissues was accompanied by decreased pulmonary ventilation and blood pressure.

The intravenous injection of sodium bicarbonate increased the alkalinity of the arterial and venous blood but the changes in arterial and venous alkalinity were frequently almost identical. This suggested smaller exchange between blood and tissue and a smaller buffering effect of the tissues on the blood. The tendency of the tissues to become alkaline on the injection of sodium bicarbonate therefore seemed to be missing. Moreover, increased respiration and blood pressure commonly occurred suggesting that sodium bicarbonate increased the acidity of the tissues.

The experiments in which the electrode was placed in tissue gave more direct information on the changes occurring in the tissues. The manganese dioxide electrode inserted between the muscle fibers of the sartorius muscle showed increased acidity on the administration of carbon dioxide. On the intravenous injection of sodium carbonate the electrode showed increased alkalinity. On the intravenous injection of sodium bicarbonate the electrode showed increased acidity. These results seemed explainable on the effect of relative impermeability of membranes to base.

This effect of impermeability of membranes was studied further by recording changes in acidity of the cerebro-spinal fluid. The administration of carbon dioxide increased the acidity of the arterial and venous blood and cerebro-spinal fluid. Intravenous injection of sodium carbonate increased the alkalinity of the arterial and venous blood and cerebrospinal fluid. Intravenous injection of sodium bicarbonate increased the alkalinity of the arterial and venous blood and the acidity of the cerebrospinal fluid. The increased acidity of the cerebro-spinal fluid was main-

tained despite a prolonged increase in alkalinity of the blood. Such findings indicated a high degree of impermeability of the cerebral membranes to base. Assuming that the membranes of or about the respiratory center function in an analogous way the acidity of the cerebro-spinal fluid serves as an index to the acidity of the respiratory center.

Towards the close of an experiment or after severe abuse entailed by the administration of low oxygen or sodium cyanide the injection of sodium bicarbonate increased the alkalinity of the cerebro-spinal fluid. It was suggested that such results are due to impaired impermeability of the cerebral membranes permitting the passage of base from the blood into the cerebro-spinal fluid. When the injection of sodium bicarbonate increased the alkalinity of the cerebro-spinal fluid it decreased respiration and mean blood pressure.

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THE EFFECTS OF POLARIZATION UPON THE ACTIVITY OF VERTEBRATE NERVE

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The purpose of the following investigation is to analyze the effects of certain extraneous physical conditions on nerve activity, first, in the light of the more recent information that has been obtained, second, by means of a more precise technique than has heretofore been applied to the problem, that of the cathode ray oscillograph. Several circumstances make precarious the interpretation of previous results.

The effects of variation in strength of stimulating shock have been interpreted too simply. Since the work of Kato, Forbes, etc., and of our own, it has become increasingly obvious that the shock stimulus may spread physically, as an electrotonic or "eddy" current, along the nerve, and stimulate at a point at some distance from the apparent locus of stimulation. This makes necessary an examination of the response under the stimulating electrode, which has not been feasible heretofore owing to the summation of stimulus distortion with physiological response. Interpretation of the effects of polarization upon the response has depended on measurements of the total energy of the action current, since the instruments used were not adequate to differentiate precisely between changes in amplitude and changes in duration. The normal changes in form are now known to be largely due to different axons in the nerve trunk possessing different properties (difference in conduction rate, irritability, refractoriness, etc.) (Erlanger, Bishop and Gasser, 1926), and variations from normal can be assigned specific meaning. With precise time measurements (limits of 0.000,02 second), it is possible to analyze shorter segments of nerve, and thus follow changes with conduction more accurately.

On the other hand, the field we have investigated has been quite completely covered with apparatus then available, and has been adequately reviewed to date elsewhere. We shall only summarize here the main points at issue, for the purpose of comparing previous interpretations with our own.

A distinction may be made at the outset between the persistent effects of a previous current, often distinguished as electrotonus (after duBois Reymond, 1849) and the simultaneous effects of current flow, termed either

polarization or electrotonus depending on the phase of the phenomenon observed. While the effect of an applied potential on a nerve may be a specific electro-chemical or polarization effect, secondary effects of like nature but of different order follow which vary with time, and these have not always been differentiated. Our experiments deal with the effects while the current is flowing, and we can see no reason for differentiation here except in point of view, between polarization and electrotonus effects so-called. The direct electrochemical change as a result of current flow through the nerve we shall refer to as polarization, realizing that similar effects may arise at a distance from the point of application through spread of current and through change of local polarization with them, and that after the potential application has ceased, electrochemical effects may persist at the point of application, and even be recorded at a distance, as electrotonus, due to secondary but similar effects of this local condition on other parts of the nerve. What the specific nature of the change in the nerve with polarization or with excitation may be we are not prepared to state, but we see no reason as yet to infer that it is different from the general physico-chemical effect of polarization potentials at a surface acting as an electrode. The simplest interpretation of nerve activity seems to be that the nerve consists essentially of a polarizable surface bounding a reactive substratum whose characteristic activity polarizes the surface to a characteristic level. Disturbance of this activity may cause a change in the polarization; and vice versa, since the surface acts as an electrode to the substratum, a change in its polarization causes a change in the underlying reaction. This is essentially the hypothesis, as we understand it, developed by Lillie (1923), who infers further that conduction takes place by the current following a potential disturbance in one region affecting the adjacent region, and thus exciting a change in the reaction at that point.

The fundamental effects of a polarizing current on nerve activity were quite thoroughly investigated by the early workers on nerve with the instruments available at that time. Our work comprises, in brief, the following phases. 1. The measurement of the amplitude and duration of the phases of the action wave within the polarized region, previously investigated particularly by Hermann (1879), and Bernstein (1912) and Gotch and Burch (1898) the latter of whom demonstrated a change in the falling phase of the potential outside the polarized area due to current passage. 2. Measurement of irritability change due to polarization (Pflüger, 1859), especially within the polarized area, as tested by the process arising under the stimulating electrode itself, without propagation, not heretofore accomplished. 3. The determination of changes in rate of propagation through the polarized area, investigated by von Bezold (1861), Rutherford (1868) and Gotch (1900), the latter of whom concluded that increase of rate at cathode compensated for decrease at anode,

leaving total rate constant; and the determination of the position of blocking of the propagated process passing through the polarized area. 4. The measurement of the effects of temperature on the shape of the action potential wave of normal and of polarized nerve; and 5, the determination of changes in the duration of the absolutely refractory period, at the stimulating electrode, with polarization, so far as we know not before investigated.

For a review of the work dealing with the nature of excitation and propagation the recent book by Lillie (1923) is most comprehensive. For reviews of the earlier work on polarization and electrotonus the discussions of Gotch in Schäfer's (1900) text, or that of Hermann (1879), cover the significant phases, later workers having for the most part only elaborated the most fundamental generalizations of the earlier group. For

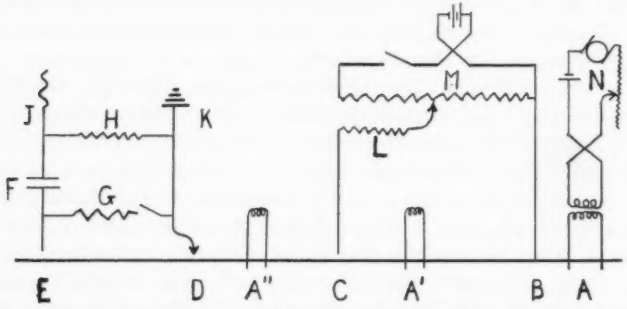

Fig. 1. Arrangement of nerve and apparatus for polarization. *A*, stimulators, *B*, proximal polarizing lead, *C*, distal, *D*, ground, *E*, grid lead. *F*, condenser (0.012 mf.) to prevent the currents applied to *CD* affecting the grid of the input. *G*, shunt resistance to decrease amplitude. *H*, leak resistance behind *F* to ground filament-to-grid current. *J*, grid, *K*, ground, *L*, 200,000ω resistance to prevent grounding.

problems involving the refractory phase, Adrian (1921) covers the subject from the standpoint of the following investigation.

TECHNIQUE. The nerve was usually arranged for the investigation of the effects of polarization as follows (fig. 1).

The unbranched femoral region of the sciatic nerve of green frog or bullfrog was mounted on three nonpolarizable electrodes which were tied around the nerve firmly enough to maintain their position without sliding. One of these, *E*, on the killed peripheral end of the nerve, was made the amplifier grid lead as usual. The other two, *B* and *C*, were made cathode and anode respectively for the polarizing current. For the amplifier ground lead, *D*, a fourth nonpolarizable electrode was mounted upon a slide through the glass door of the moist chamber, in such a manner that it could be raised off the nerve, and slid along its whole length, grounding the nerve at any point desired. It could be manipulated entirely without

opening the chamber. This general scheme was modified as occasion arose, as for instance, when E was made one polarizing lead, or one of the stimulators was made the ground lead. The polarizing circuit could be worked either through the commutator, synchronized with the stimulus, or acting as a stimulus itself, or through a hand key for a steady current. It is obvious that this polarizing circuit could not be grounded without acting as an amplifier ground, and therefore had to be balanced for resistance and capacity when used with the rotating commutator, to avoid distortion. (When made by a hand key the condenser at F prevented more than a momentary disturbance of the amplifier.) Also, when leading via D, from any point above C toward B, the polarizing circuit tends to ground the nerve through both B and C, or by whichever pathway has the lowest resistance to D, making a complicated record of the action current. (Bishop, Erlanger and Gasser, 1926b, discuss such complicated records.) A resistance high compared to the nerve resistance between B and C was therefore inserted in series in the polarizing circuit, and the voltage increased correspondingly (up to 30 volts on a rheostat as a potentiometer). The stimulators A, $A,'$ $A,''$ could be connected to a single coreless Porter coil worked through the rotating commutator, giving from 5 up to 30 shocks per second, as desired.

The remainder of the apparatus has been described heretofore. Our rotating interruptor has been replaced by an entirely new one, modelled upon the machine previously described, but of much better design and execution, worked out by Mr. John H. Zimmer in the shop of this department. The outstanding feature of this apparatus is the elimination of hard rubber insulating half sections on the commutator wheels, which have been replaced by insulated bronze dead segments, with narrow air or mica filled gaps. This feature eliminates uneven wear and jumping at the gaps, and irregularity of contact due to dirt under the spring contacts from erosion of the rubber, which was a constant source of annoyance in the old machine. The apparatus runs with no appreciable vibration, can be adjusted with extreme accuracy, and adapts itself readily to any connections demanded for our circuits. Without this instrument of precision many of our more recent experiments could not have been performed with significant accuracy.

1. Anodal and cathodal effects on amplitude. We can corroborate fully the finding of former investigations that in the region of the anode, an action current is of greater amplitude, in the region of the cathode, of smaller amplitude, than the normal action current of a given nerve unpolarized, if the polarizing current is not too strong. This is not due to differential effects on the axons with conduction, for it may be observed at a lead from the stimulating cathode. The threshold is uniformly high at the anode, the reverse at the cathode, in all our experiments with weak

currents according to expectation. Given a just maximal stimulus for α, the supernormal amplitude at the anode and subnormal amplitude at the cathode occur whether the action wave travels into the region in question, or originates there, and regardless of the direction of polarization or of conduction (fig. 2). This is evidence that the action current is characteristic of the condition of the nerve locally, and does not arrive at its state because of increment or decrement from normal nerve; as Kato, and Davis, Forbes, Brunswick and Hopkins also decide for narcotized nerve. When the polarizing current is progressively increased above that causing the maximum amplitude increase under the anode, the amplitude is re-

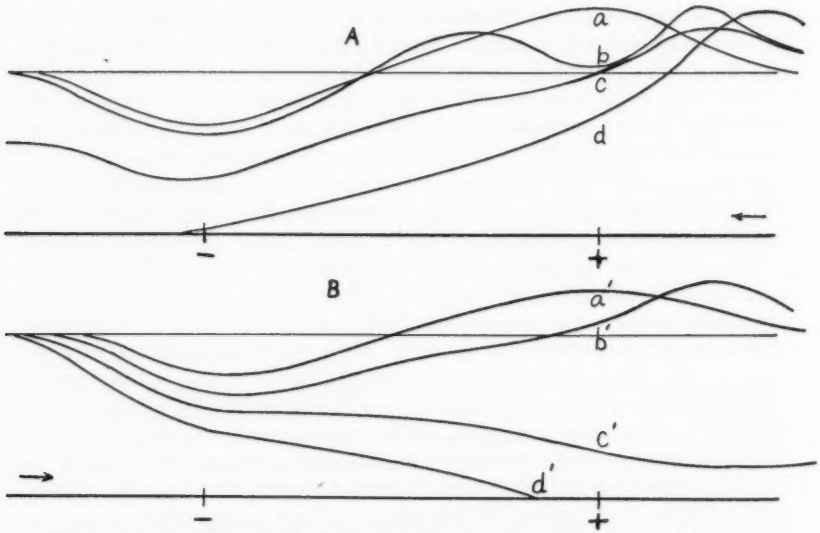

Fig. 2. Amplitude in polarized area at different potentials. *A*, anode proximal to stimulator. *B*, cathode proximal. *a*, weak, *b*, medium, *c*, partial block, *d*, total block under distal polarizing electrode. *a′*, *b′*, *c′*, *d′*, same for reverse direction.

duced until the wave disappears entirely. By the time the amplitude *at the anode* has been reduced to normal or slightly below, the region of supernormal amplitude has moved into the polarized area, toward the cathode, where it is in general not quite so pronounced as it was when at its maximum at the anode itself. There is also a region of supernormal amplitude distal to the anode. As the current increases, this area of increased amplitudes spreads to either side of the actual anode, though at the anode itself, the amplitude is decreased.

When the nerve at the anode is depressed sufficiently to reduce the amplitude at the anode to about normal, the supernormal effect beyond,

toward the cathode, seems to be due to recovery of the action potential from the depression, to the usual anodal maximum. It might also be affected, however, by the greater slowing of rate in the faster conducting fibers than in those which normally conduct more slowly, as the former fibers are in general affected by stimulating currents more readily than the latter. This slowing in faster fibers would tend to bring their action waves back under the crest of the whole group, and thus raise the amplitude. Stronger polarization would counteract this by affecting all fibers, and possibly blocking some altogether. At the strength which causes a maximum on either side the anode proper, this process would thus have taken place twice, at each of two maxima, the intervening anodal depression being due to partial blocking or to slowing of all fibers alike in a region of greater than optimal polarization for the most susceptible fibers. The above explanation, however, by no means applies to the central fact of increase in amplitude at the anode, and decrease at the cathode, for this takes place in the axon action currents, as recorded under the stimulating electrode.

With slightly more current, the amplitude in the whole interpolar region falls off increasingly to the cathode, or to extinction short of it, showing no node of increase in this area. In this case no recovery is in evidence. If the action wave passes the cathode at all, it increases slightly in amplitude in the normal nerve beyond, but not necessarily to normal amplitude. If currents are used to polarize sufficient to depress the action wave at the anode to below its supernormal maximum, injury is rapid, and recovery slow, or failing altogether. The stage at which increase of amplitude passes into depression, or blocking, may be observed by recording the action current after it has passed both anode and cathode. (Fig. 1, stimulus at *A*, anode *B*, cathode *C*, lead *D* placed between *C* and *E*.) The amplitude beyond the cathode returns approximately to normal as long as the amplitude at the anode, or between anode and cathode, is normal or above. Further reduction at the anode therefore may involve *blocking* of some fibers, for when the nerve does not show the supernormal anodal effect where its action potential wave arrives at some optimally polarized point between anode and cathode, recovery is also incomplete after passing the cathode; i.e., some fibers have failed to conduct past the polarized region. We have some evidence to indicate, however (see under conduction), that actual blocking of conduction takes place first toward the cathode rather than under the anode.

Figure 2 presents diagrams based upon measurements of action potential amplitudes along the nerve, with different strengths of polarizing current. All measurements are reduced to proportion of the normal for the nerve at that point, eliminating the effect upon the normal amplitude of variations in nerve condition, differences in resistance, etc., and leaving only the

effect of the flow of polarizing current. Curve *a* represents the amplitude at a strength of polarizing current that gives a maximal increase, at the anode, propagation being as diagrammed in figure 1, stimulus at *A*. *B* anodal. In *b*, this increase has been counteracted at the anode by the effect of a stronger current; pushing the anodal node toward the cathode, and showing a second supernormal region distal to the anode where due to spread, the anodal polarization may be considered to be at an optimum for the increase. No blocking takes place under these conditions. In curve *c*, the second hump disappears, and the anodal region seems to be a pure decremental area as far as amplitude is concerned, depressing certain action currents to extinction, as is shown by failure of recovery below the cathode. In this case the effective decremental area is so wide that it extends across to the region of cathodal depression. Apparently any fiber which conducts into the cathodal area at this stage, conducts through this area, for the amplitude increases beyond the cathode at about the same rate as it decreases toward the cathode. In curve *d* the above process is carried to a further stage. At this strength the cathode seems to block the decremental action currents that pass the anodal region, in spite of the higher irritability that might be expected here. In fact this current is probably strong enough to *depress* the irritability of the cathodal region, in accordance with Pflüger's findings. The polarizing currents used for these curves were roughly in the proportion 2 to 3 to 5 to 12 for *a*, *b*, *c*, and *d* respectively. With the strong currents the nerve rapidly deteriorates, and the above diagram is a composite of a number of different sets of results from different nerves, with many duplications and overlappings. The actual strength of current required is of course dependent upon size of nerve, length between electrodes, specific resistance of nerve, etc. Usually, the increase of the anode persists until the polarization has depressed the corresponding cathode response to about one-half normal value.

When the direction of polarization is reversed (in fig. 1, *B* cathodal, *C* anodal), the results are similar to the above, until we come to fairly strong currents (as in *c'* and *d'*, fig. 2). At a polarizing strength such that an action wave passing decrementally through an anodal area will partially recover after passing the cathode, showing that the cathode is not totally blocking the anodally decremented wave, the disturbance passing in the other direction is reduced in amplitude at the cathode, and then undergoes an irrecoverable depression under the anode, emerging into normal nerve lower than in the reverse direction. That is, fibers which conduct action currents from a normal into an anodal area will then conduct past a cathode, and recover, when after conducting from a normal through a cathodal area, they are blocked by the corresponding anode. In spite of higher irritability at the cathode, in other words, an action potential wave conducted through it is less able to pass the anode than a

wave conducted into the anode from normal nerve; while in the reverse direction, even an apparently depressed action current is able to pass from an anodal region through the cathode. This indicates that the elicitation of an excitation at a given point depends both upon the irritability of that point, and also upon the intensity of the reaction in the adjacent nerve, *as measured by its potential.* To be sure we have produced a physiological gradient in the nerve, but this gradient should be of the same form when reversed, whereas the results are different. Conduction rate data (see below) indicate a similar state of affairs.

In fatigued nerve, or nerve left for some time in normal salt solution, the relative effects at anode and cathode tend to vary from the above. The blocking at the cathode becomes easier, compared with the anode, and the increase of amplitude at the anode extends further toward the cathode. It appears that the effects of current flow are then less injurious at the anode, and more so at the cathode. Taken with the observation that the demarkation potential of nerve removed from the body tends to decrease with deterioration of the nerve, and that cathodal polarization opposes the demarkation potential while anodal polarization adds to it, the inference may be drawn that with lowered potential throughout, the anodal effect will be partly dissipated in raising the anodal region back to a normal level, while the cathodal effect will be more severe because opposed by a lowered potential in the nerve itself. In this discussion it is obvious that "polarization" cannot be limited to a mere shift of potential, as in a condenser charge, but that it involves an altered reactivity of the nerve processes as a whole, which presumably go on at the seat of the essential axon polarization, and of whose reactivity the nerves' polarization potential is an external sign. With deterioration of the nerve, permeability presumably increases, and the nerve's resistance to change correspondingly decreases, as in other tissues, and this would operate in the same direction.

When the nerve is polarized through electrodes placed upon its two killed ends, the general effects are the same as when the electrodes are placed on the live sides, except that from 3 to 4 times the potential drop per centimeter is necessary to produce the same effect, although the lengthwise resistance is less than the crosswise. If the nerve is a core conductor, enclosed in a polarized membrane, as conventionally represented, the cross polarization should not be altered by this method of polarization, since it is the case of parallel conductors of uniform but different resistances, each having the same potential drop; and the question may be asked why this should affect the rate or amplitude, at all, away from the vicinity of the electrode, where the nerve is injured anyway. Polarization from the sides of the nerve of course gives two components of potential drop, one across the axons and one along them, and it is the cross polarization that might be expected to be the effective one if the nerve corresponds to the con-

ventional theory regarding it. At the killed end, however, the sheath may still be polarizable, when the spread of polarization potential along the nerve may accomplish cross polarization of the axons. Possibly both components have effects, and the conditions at the nodes of Ranvier might lead one to anticipate an effect of some kind from lengthwise passage of current. At each nodal constriction there must tend to take place a redistribution of current between inside and outside of axons, with a consequent cross-polarization at the node. Some of our experiments might be more easily interpreted on that assumption, but at present we have not gone far enough to state the conditions definitely.

Decreased amplitude at the cathode, therefore, seems to be due to a real decrease in the amount of axon activity with each excitation, even though that activity is easier to set off by a stimulus. Increased amplitude at the anode must connote an increase of activity in the axons, which perhaps accounts for the severity of the effects of anodal polarization on the nerve, which rapidly deteriorates with anodal polarization. *Decrease* of amplitude at the anode with stronger polarizing currents may be due in part to blocking of stimulation, only part of the axons being capable of response, or of conduction. In each case each axon may discharge maximally for the given condition of the nerve, as in normal unpolarized nerve, the quantitative value of the response depending upon the metabolic level of the nerve, as affected by the polarization.

2. Irritability within the polarized region. At their face value, our results are in complete agreement with previous work on irritability, that polarization decreases the threshold at the cathode, and increases it at the anode, for weak or medium currents, while the stronger the current the further the anodal increase of threshold encroaches upon the cathode, until with strong currents the cathodal threshold is increased. It occurred to us that since a normal action wave may travel past one pole to be blocked by a second, an action wave initiated within the area might be blocked before it emerged, giving the appearance of high threshold where the threshold was really low. For this reason we have measured the relative irritability to break shocks at the site of stimulation, by placing the ground lead at or near the stimulating cathode. The results could be anticipated from the diagrams, figure 2. With weak or medium currents, since the action waves are not blocked, but may be recorded outside the polarized area, the results are the usual ones. With strong currents, a process may be excited but be unable to propagate to an appreciable distance, or may do so only with partial blocking. *Near* the cathode, in the interpolar region, when a process can be initiated it will propagate *toward the cathode*, but may decrease rapidly to extinction toward the anode. If it passes the

cathode, even though of low amplitude, it increases in the normal nerve beyond.

These results only confirm the former findings, except that they contribute to a closer analysis of the relation between irritability and conduction. It may be pointed out here that where irritability has been tested by recording an action current after propagation, the data applying to irritability at the anode have indicated a higher threshold for strong currents than presumably existed, without, however, changing the order of the events materially.

3. Changes in conduction rate through a polarized region. In the measurement of conduction rate within the polarized area we met with certain difficulties in collating the data. First, it was difficult to get a complete series on one nerve, because the treatment was rather severe. But it was difficult to compare two nerves of different irritability, rate and resistance, when a series was run in two experiments. Then, since the regions in which significant changes in rate took place were necessarily small, a slight error in measurement made an enormous error in rate. The following procedure was finally worked out.

A strength of current was chosen as a "weak" current, which caused a maximal increase in amplitude at the anode, of an action wave passing into it, and a slight decrease at the cathode, and allowed complete recovery to normal beyond. For the rest of the experiment amplitudes were ignored to expedite progress. From the beginning of the line on the face of the Braun tube, one operator stepped off with dividers the distance to the first upward inflection of the foot of the action wave, and pricked this off on coördinate paper. The other operator meanwhile read the position of the ground electrode on the nerve, and set the apparatus for the next reading. To read the position of the electrode, a glass millimeter scale was cemented to the glass door of the moist chamber, a small light was held to illuminate both nerve and the observer's eye, and the scale was read when the reflection of the observer's pupil from the glass door fell upon the edge of the electrode contact nearest the grid lead. With this technique a satisfactory series could be made before the nerve showed appreciable injury, while accuracy of readings lay within 0.2 mm. The chief source of error is in estimating the start of the process rising asymptotically from the base line.

The results indicate that the modifications of amplitude and of rate due to polarization hang together in a quite consistent manner, as shown in figure 3. With the anode proximal to the stimulating electrode, as the amplitude increases when a propagated wave reaches the anode, the rate decreases, and as the amplitude decreases under the cathode the rate increases. Our records generally show a rate, just as the wave approaches

the cathode, actually higher than normal, often considerably higher (Rutherford, loc. cit.).[1] Further, from calculation of the rate of the start and of the crest of the action wave, it may be shown that since the start reaches the anode first, and there slows up in conduction, the wave as a standing wave plotted along the nerve shortens up, and the potential gradient becomes steeper, as the wave approaches the anode, though the actual time to maximum at a given point increases; and the wave lengthens

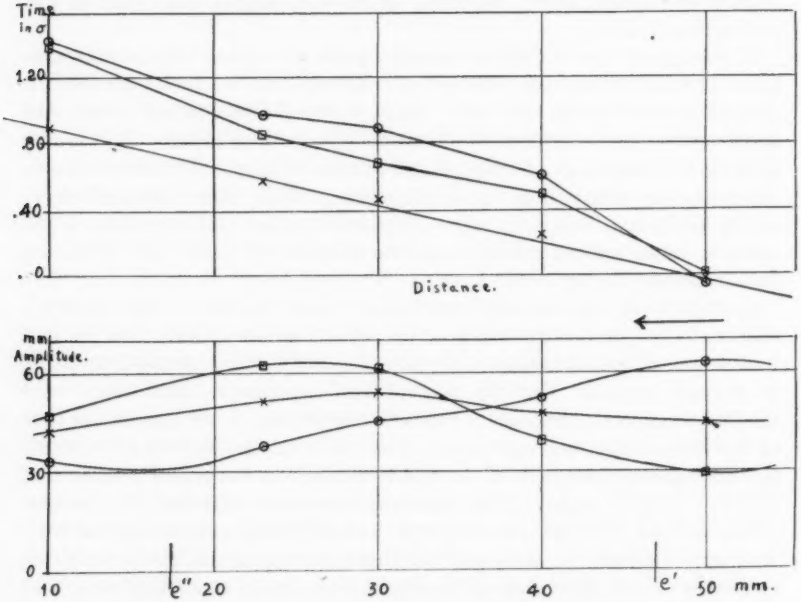

Fig. 3. *A*. Rate of start and amplitude, anode proximal to stimulator ⊙, cathode proximal, ⊡, normal, *X*. *e′*, *e″* indicates position of polarizing electrodes. The slope of the curve measures the rate, the latter being greater where the curve is flatter. Bullfrog sciatic, expt. 5/25/24. Weak currents. Time in sigma.

out to more than normal as it passes the anode, both as a standing wave or distance-potential curve, and as a time-potential curve at a point. The rate is slower in spite of both increased potential and steeper gradient.

[1] Rutherford (1868) reports that, stimulating just peripherally to the polarized area, and recording the muscle contraction, the rate is increased near the cathode and decreased near the anode. He did not test the nerve by shorter segments, and made no study of the conditions between the polarizing electrodes. He studied the region below the electrode in question (between the polarizing lead and the muscle), and measured the conduction, only after the cessation of fairly strong polarizing currents, not during the current flow.

This constitutes evidence that, once the stimulus is adequate, the character of the action wave is determined in the main by the conditions locally in the nerve, and not by a decremental or incremental process due to the intensity of the reaction elsewhere. The reverse process takes place under the cathode.

This effect of slowing at the anode is so pronounced with strong polarizing currents as to lead to the inference that blocking under the anode may

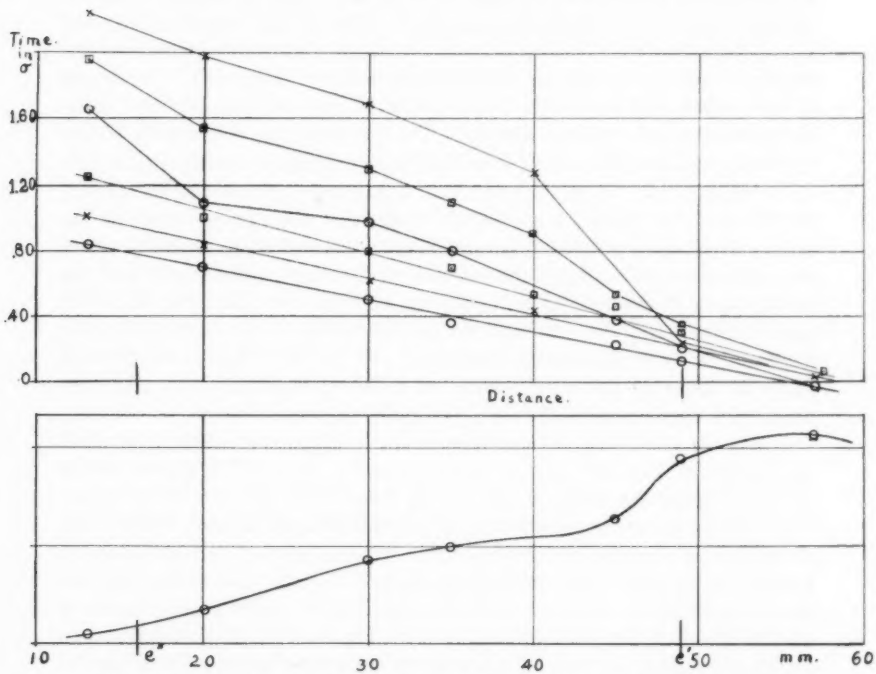

Fig. 3. *B.* Rate of start and amplitude, same as above, except as follows; anode proximal, ⊙, cathode proximal, *X*, strong current; cathode proximal, weak current, ⊡. Normals correspond in markings. The differences in the normals are due to the effects of current during the experiment, which are cumulative, and gradually slow all the rates. Bullfrog sciatic, expt. 5/23/24. Time in sigma.

be due to reduction of velocity to zero, even as the amplitude increases, while blocking under the cathode may be due to reduction of amplitude (i.e., intensity) to zero, although the velocity increases at least at first. The apparent reduction of amplitude under the anode is accompanied by the progressive separation out of axon processes, and may be due to this and not to a real decrease in amplitude of the axon processes. It may be

argued that the decrease *at* the anode with medium currents, with an increase beyond the anode (toward the cathode), discussed above, indicates that blocking would result *at* the anode from further increase of current. But the fact of an increase between anode and cathode following a decrease *at* the anode implies, as would be expected, that the anodal intensity of polarization decreases toward the cathode, which should allow the partial recovery at least of any axon action process that was depressed in passing the anode at all. That blocking at the anode, even with polarization that seems to depress the amplitude, does not take place, is indicated by the fact that the action process is completely obliterated first near the cathode, and only by still stronger currents at the anode itself. The nature of the increase in amplitude between anode and cathode has been commented upon in a previous section. The circumstance that finally blocks the action potential appears to be failure to propagate, rather than failure to develop locally when initiated. It would appear that the irritability of the nerve decreased more rapidly than the stimulating effect of the augmented action potential increased, until the following region of nerve can no longer be excited by the reaction in the nerve preceding it, and the action wave therefore dies in its tracks, at whatever point the threshold cannot be reached, even though the preceding nerve may have undergone a normal or even supernormal response. Since this happens in different fibers at different positions, owing to different thresholds, etc., of the axons, the appearance is that of a gradually decrementing wave.

When the polarizing current is reversed, making the cathode proximal to the stimulating cathode, a striking modification of the above results occurs. Under the anode as before, the rate is slowed. Under the proximal cathode, contrary to expectation, the rate is again slowed, though not as much as under the proximal anode. This occurs without exception in all our experiments, with the same strength of current that in the reversed direction causes an increase of rate considerably above normal under a distal cathode. This cannot be laid to the previous effect of anodal polarization persisting, for it occurs in records where the cathodal polarization was produced in a fresh nerve, and it does not occur distally even after the distal region has been anodally polarized. What it amounts to is this: that an action wave of normal rate and amplitude entering a cathodal region of a given degree of polarization is decreased in amplitude and its rate is slowed, but when the action wave of increased amplitude and subnormal rate enters a cathode from an anodal region, the wave is decreased again in amplitude but its rate is increased. This occurs in spite of the fact that at some region between the polarizing electrodes the amplitude must have been normal, and at some region (possibly not the same point), it must have propagated at normal rate.

Either of two conditions might be pictured to account for this. There

may be a real decrement taking place, that is, the propagation may depend in part on the amplitude or potential gradient of the action wave in the previously excited region, as the Lillie theory demands; then a higher amplitude, anodal, might cause a faster rate into a region of higher irritability, cathodal, but a normal amplitude ought also to cause a faster rate into a region of higher irritability (proximal cathode), which it fails to do. We would have to make the further assumption that the cathodal decrease in amplitude (stimulating value of the propagating wave), is relatively more effective than the cathodal increase of irritability, when passing from a normal to a cathodal area; but that when passing from an anodal area with increased amplitude, the same increase of irritability as before allowed a faster conduction, due to the high stimulating value of the anodally increased potential. This is consistent with the fact that in the anodal region, in spite of higher amplitude, low irritability and slowed conduction appear.

The other condition that seems possible is that, while reversal of current should exactly reverse the conditions of cross polarization of the nerve, which may be considered as the occasion for the amplitude changes, the lengthwise direction of current is also reversed with respect to the direction of propagation. If the longitudinal component of current effect could be demonstrated to be a cause of the change of rate of propagation, there might be expected a general slowing, under both anode and cathode, with one direction of polarization more than with another. This total slowing does not appear to be marked, if it exists. The question must be left open for the present.

As the action wave passes the distal cathode, after having speeded up as it approaches it, the rate is again slowed, as if the low action wave from the cathode were working "up hill" into the normal region of higher threshold. As it passes a distal anode the rate is slowed less than the above, but is still below normal. The slowing in both cases is apparently at, and distal to, the electrode; i.e., it is beyond the polarized point proper though the effects of polarizing current may be presumed to spread to some distance beyond the electrode. In general, it appears that whenever the process travels from a region showing a high amplitude of wave to one of lower amplitude (normal or polarized), the rate may be expected to increase, and vice versa, as if the lower amplitude were associated with lower threshold and higher amplitude with greater stimulating power for adjacent nerve. The process therefore speeds up when it runs "down hill," etc. Exceptions to this rule may be hypothetically explained if we assume that the decrease of threshold, with low amplitude, is relatively less than the decrease in stimulating value of the propagating disturbance.

4. The effect of temperature and polarization on duration of phases of activity. a. Conditions of experimentation. In view of the depressing

action on nerve of polarization, we have undertaken to compare with this effect the effect of temperature changes on different aspects of the propagated disturbance (amplitude, time to maximum, total duration, and refractory phase). It was observed that the shape of the action wave changed so rapidly with conduction away from the polarizing electrode that records at any point except at or close to the locus of stimulation were not conclusive. We have already described an arrangement for observing the action current elicited by a galvanic stimulus, by balancing out the galvanic current in a wheatstone bridge of which the nerve forms one arm (Bishop, Erlanger and Gasser, 1926a). Owing to the different utilization periods of different axons in the nerve trunk, a constant current is not a suitable stimulus for obtaining the true shape of the resultant excitation wave, for the axons are stimulated out of phase with one another. The induction shock gave a transient "escape" distortion even when the nerve was put into this bridge, that overlapped the start of the action current. An arrangement was therefore resorted to partially bridging out this effect, as described elsewhere (Bishop, Erlanger and Gasser, 1926b), and the lead in some cases was two or three millimeters distant from the cathode of the shock, this distance of conduction being insufficient to materially alter the wave form, but greatly reducing the escape. The amplifier ground lead could be placed at or close to the cathode of the shock, and was made the cathode or anode, at will, of the polarizing circuit, with the grid lead at the killed end as usual. The action wave at the locus of stimulation, which was also the site of polarization, when stimulated by a shock whose effective duration was 0.04 to 0.06 σ must have had its constituent axon action waves almost exactly in the same phase.

The ideal conditions for such a lead are, high irritability, which requires only a moderate stimulus; a small nerve, so that all its component axons will be stimulated at the same point under the electrode; absence of a sheath, which when present acts as would a broad contact of the nerve with the lead; and a more or less uniform composition of the nerve, i.e., absence of β and γ waves. Such conditions are satisfied in the green frog sciatic VIII motor root, and in the dog phrenic with the sheath dissected off, giving us a "cold-blooded" and a "warm-blooded" nerve. Changes of temperature in these nerves cause transient effects that are recovered from (Broemser, 1921) and above 30° the frog sciatic failed to recover completely, though records could be obtained beyond this figure. Polarization currents were considered as weak if they produced the maximal increase at the anode above the normal unpolarized condition (see fig. 2); as medium if they reduced the action potential at the anode to normal amplitude or slightly below; and as strong, if they reduced the anodal action potential to about one-half its normal amplitude or less. For each strength of polarizing current, the stimulus was made just maximal for the

given anodally polarized condition of the nerve under the leads, and this stimulus, though supermaximal for the cathodally polarized and unpolarized conditions, was used throughout a set of observations. The records consisted of contact photographic prints of the oscillograph picture from break induction shocks, with the locus of stimulation unpolarized, anodally polarized, and cathodally polarized.

b. *Effects of polarization on duration.* The consistent effects of polarizing currents in all cases studied were substantially as in figure 4 (green frog sciatic, 1, 14, '25 plotted in linear coördinates). 1. For weak anodal polarization there was an increase of amplitude of $\frac{1}{4}$ to $\frac{1}{2}$, an increase of rising phase of 0.33 to 0.43 or 0.10σ or 130 per cent; and an increase of duration of the falling or restoration phase of 1.29 to 2.22σ, or 0.93σ, 172 per cent of the normal duration of restoration. 2. For weak cathodal polarization, there occurred a decrease of amplitude of $\frac{1}{4}$ to $\frac{1}{3}$, an insig-

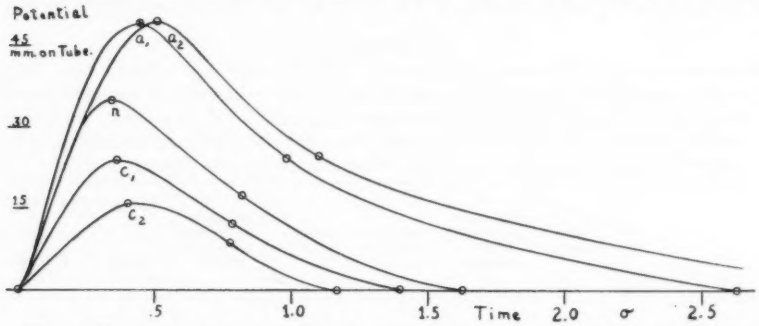

Fig. 4. Shape of action waves at polarized electrodes, recorded at the stimulus. This was also the proximal polarizing electrode, made anode or cathode. Normal, n. Anodal, weak, a_1. Cathodal, weak, c_1. Anodal, medium, a_2, Cathodal, medium, c_2. Expt. 1/14/25 green frog sciatic. Drawn on linear coördinates from measurements of logarithmic time values of oscillograph. Time in sigma.

nificant increase of duration of rising phase of 0.33 to 0.34σ and decrease of duration of the falling phase of 1.29 to 1.06σ, or 0.23σ, 82 per cent of normal.

In these cases the time to the crest or maximum was not greatly altered, though stronger currents in either direction increased the time to maximum progressively; nor did the time of the start during the induction shock vary within the error of measurement (0.02σ). 3. With medium anodal polarization, the rising phase was very slightly prolonged, the falling phase much more prolonged than with weak currents, and the amplitude was sometimes reduced below normal, even when the stimulus was increased to a new maximum. 4. With medium cathodal polarization, the rising phase was generally slightly prolonged, the falling phase more shortened than with

TABLE 1

Measurements of action waves of figure 5A, to show change with temperature

	RISING PHASE	PER CENT OF NORMAL RISING PHASE	TOTAL DURATION	FALLING PHASE	PER CENT OF NORMAL FALLING PHASE	PER CENT OF VALUE AT 35°	
						Rising phase	Falling phase
25°							
Normal	0.22		1.13	0.91		110	240
Anode	0.27	122	1.35	1.08	116	122	177
Cathode	0.24	109	0.96	0.72	80	140	190
35°							
Normal	0.20		0.58	0.38			
Anode	0.22	109	0.83	0.61	160		
Cathode	0.17	85	0.55	0.38	100		

TABLE 2

Measurements of action waves of figure 5B, to show change with temperature. Even at 34°, two degrees higher than the initial temperature, the action waves were longer in duration than at 32°, due to having been maintained at 24° for a time. This time lag for adjustment to changed temperature appears to be slower for the falling phase however than for the rising phase, which has shortened to the expected degree

	RISING PHASE	PER CENT OF NORMAL RISING PHASE	TOTAL DURATION	FALLING PHASE	PER CENT OF NORMAL FALLING PHASE	PER CENT OF VALUE AT 32°	
						Rising phase	Falling phase
32°							
Normal	0.27		1.12	0.85			
Anode	0.31	115	1.46	1.15	135		
Cathode	0.27	100	0.92	0.65	76		
24°							
Normal	0.42		2.04	1.62		155	190
Anode	0.46	110	2.30	1.84	114	148	160
Cathode	0.46	110	1.98	1.52	94	170	233
34°							
Normal	0.26		1.18	0.92			
Anode	0.31	119	1.50	1.19	130		
Cathode	0.21	81	1.14	0.93	100		

weak currents, and the amplitude considerably reduced. The position of the start was not altered in either case. 5. With strong anodal, and 6, with strong cathodal polarization, the results of 3 and 4 were merely accentuated, until finally very low action currents were elicited only from enormous shocks.

c. *Effect of temperature on polarization.* We have undertaken to compare these effects of polarization with the effects of temperature changes on different aspects of the propagated disturbance (amplitude, time to maximum, total duration). Most reactions have a positive temperature coefficient, and the above comparison should tell us whether an apparent depression were really that, or the acceleration of some opposing factor. It was found that increase of temperature above normal shortened both the rising and falling phases of potential, cooling lengthened them. The

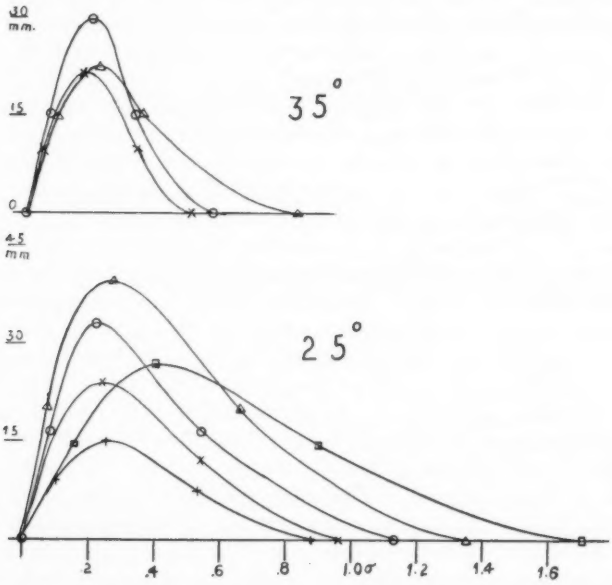

Fig. 5. *A.* Polarization at different temperatures. Normal, ⊙. Anode, weak, △. Cathode, weak, *X*. Anode medium, ⊡. Cathode medium, +. Linear coördinates. Recorded after conduction from a point 2 mm. outside polarized area. Green frog motor root, 1/12/25.

greater effect was on the falling phase (recovery). The results, however, are complicated by changes with time (Broemser, 1921) and by the possibility that processes underlying rise of potential and recovery may interact mutually. The data seem to indicate that factors in the nerve's activity underlying the rise and the recovery phases respectively may have different temperature coefficients, the latter having the larger coefficient. Results for a green frog motor root (the action waves replotted in fig. 5 A to linear coördinates), are collected in table 1 including the effects of polari-

zation at different temperatures. Data for a dog phrenic (fig. 5 B) appear in table 2. The temperature coefficients for the durations of the two phases measured over ten degrees show a fairly consistent difference. The experimental error in measuring start, crest and end of the wave is large, and especially large relative to the duration of the time to maximum, and these figures show considerable variability.

Measurements of amplitude indicate that warming the nerve first increases the amplitude, but higher temperatures may decrease it. This

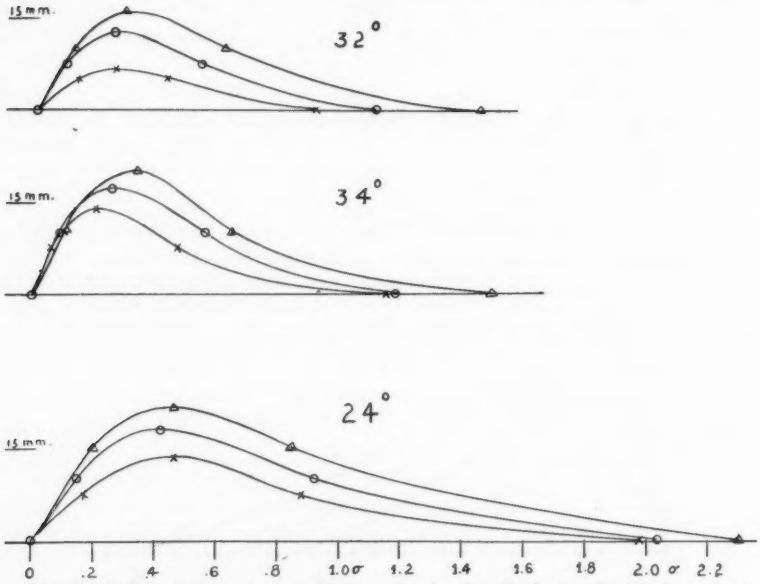

Fig. 5. *B*. Same as above, normal, ⊙. Anode, △. Cathode, *X*. Weak currents. The records at 32° were made first, then those at 24°, then at 34°. The nerve had not yet reached equilibrium condition at 34°. Conducted from a cathode 3 mm. outside polarized area. Dog phrenic. 1/10/25. Time in sigma.

may be due either to irreversible changes in the nerve, or to the cutting short of the rising phase by a very abrupt recovery, since the recovery is affected by temperature more than the rising phase in general. It may be that a recovery process sets in actually before the attainment of maximum potential, but starts slowly. Accelerating the recovery should then not only lower amplitude, but should also shorten the apparent duration of the excitation or rising phase, though the process underlying the rise may also be going on during part of the recovery period. If the overlapping of the two processes is about symmetrical with respect to the potential crest, the

differences in the two phases should be real ones. The further change that takes place with time after the temperature has been altered was not sufficiently checked in these experiments to determine the degree of accuracy of these data.[2] In the case of the green frog root, lowering the temperature from 24° to 17° depressed the potentials to such an extent that their phases could not be measured with significant accuracy. With time they would undoubtedly have become higher at this temperature. If the actual potential amplitude as observed is a resultant of two opposing activities, excitation and recovery, having different temperature coefficients of intensity, changes of amplitude with temperature should be complicated by the changing relations of these variables.

Having altered the form of the wave by temperature change, the effects of polarization upon the form show no significant differences due to the temperature, aside from this general shortening with temperature increase; that is, the differences are within the variations from nerve to nerve, which we find rather large under the conditions of our experiments.

Lillie (1923) finds in the iron wire model that the temperature coefficient (Q_{10}) of rate of transmission is about 1.4 to 1.5, while that for complete recovery or passivation (non-refractoriness) is about 3, thus distinguishing two successive reactions in the mechanism. Lillie also points out, as we have, that the different effects of temperature on different nerve phenomena suggest separate reactions of nerve discharge and restoration.

5. *Effect of polarization on absolutely refractory state.* Finally, we may refer to Adrian's (1921) findings that on cooling, the refractory phase increases in duration with the increased duration of the action potential, and vice versa for warming. For further comparison of the two states of cooling and anodal polarization, we have determined the effect of polarization on the duration of the absolutely refractory state.

A pair of platinum electrodes was connected with two coreless Porter coils in the secondary circuit of each of which was inserted a resistance of 100,000 ohms, to prevent shunting of the nerve stimulus from one coil through the other. The cathode of this stimulator was connected to the polarizing lead nearest the grid lead at the killed end of the nerve, and the cathode was also made the nerve ground lead for the amplifier. The nerve was polarized from a potentiometer through 200,000 ohms, as described heretofore (fig. 1, A, A', C and D superposed). One of the secondary coil circuits was balanced to eliminate distortion (Bishop, Erlanger and Gasser, 1926b); the nerve was then stimulated by a strongly supermaximal shock from the other coil, followed by a similar shock from the balanced coil so

[2] Since this work was done, a further experiment by Gasser and Erlanger shows that the differences in temperature effects upon rising and falling phases is a real one, and appears to be even more striking after allowing the nerve to come to a state of equilibrium at the changed temperature.

timed that the nerve was just refractory. No change was observed from considerably increasing the second stimulus. The time of this event was observed as a dial reading on the worm gear that moves the interrupter brush. The nerve was then polarized, and the shift of the interrupter brush recorded that brought the second shock just into the new refractory position. With anodal polarization the end of the absolutely refractory phase was not only relatively earlier, from the end or the prolonged action wave characteristic of anodal polarization, but slightly earlier absolutely, as measured from the beginning of the wave. With cathodal polarization the reverse was true, the refractory phase lasted longer both absolutely, and relatively to the total duration than in the normal unpolarized condition. The effect of cathodal polarization was the more pronounced. These data are collected in table 3 (green frog sciatic, 1, 28, '25).

TABLE 3

Change in duration of the absolutely refractory period with polarization. The stimuli were applied at the polarizing electrode, the lead was made down the nerve to allow the response to increase in size with conduction. Values of polarizing current in arbitrary units, the weakest being very weak, no injury resulting from the strongest. Time of end of absolutely refractory period in sigma, computed from movement of brush on interrupter wheel. 0.01 sigma represents 6 degrees of arc on worm gear dial

POLARIZING CURRENT STRENGTH	TIME IN σ		POLARIZING CURRENT STRENGTH	TIME IN σ	
	Anode	Cathode		Anode	Cathode
5	−0.045	−0.02	10	−0.03	+0.05
10	−0.027	+0.03	10	−0.03	+0.04
15	−0.025	+0.07	20	−0.03	+0.19
20	−0.025	+0.18	10*	−0.02	+0.02

* Increased shock strength.

Effect of polarization on relatively refractory state. In view of the fact that cathodal polarization in general lowers the threshold, although it lengthens the absolutely refractory phase, we further investigated the return of irritability after excitation during the relatively refractory stage as affected by polarization, to determine whether the intervention of refractoriness abolished the cathodal increase of irritability. The first procedure was to stimulate a nerve by a constant current from the rotating interruptor wheel, at a strength to cause all fibers to react almost at the start of the current, i.e., to be in approximately the same phase, and to allow the current to continue flowing throughout the duration of the action wave, thus polarizing the nerve cathodally. An induction shock having a common cathode with the galvanic current was then employed to determine the irritability at different times during and after the potential wave, and the resultant disturbances observed from leads further down the nerve

when the two processes had been allowed to separate out due to differences of conduction rate, the second impulse traveling slowly in the partially refractory wake of the first (Gotch, 1910) (Gasser and Erlanger, 1922). The time of the events could also be recorded at the site of stimulation.[3]

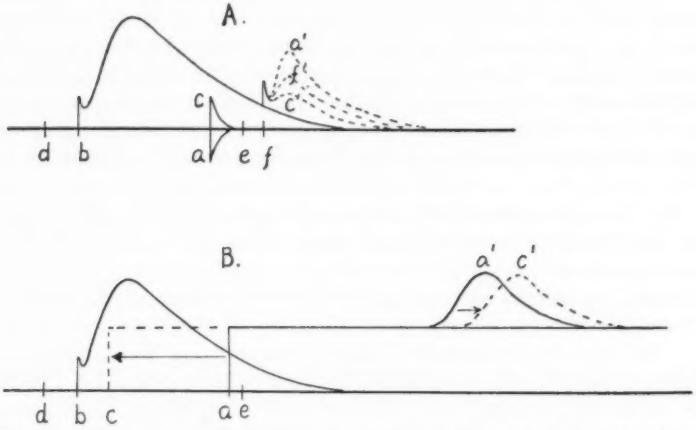

Fig. 6. *A*. Diagram of action waves as seen on the oscillograph, showing the effect of a shock which does not stimulate, in the absolutely refractory stage, upon the response to a subsequent shock in the early part of the relatively refractory stage. $a + c$, anodal and cathodal shocks in the absolutely refractory stage ending at e due to excitation started by shock b. f', a' and c', second responses, to shock at f, and to f plus a and f plus c respectively.

B. Same, for effect of galvanic current. When more current flows during the absolutely refractory stage, c to a, the time of the second response, to the galvanic current, is later, a' to c'.

These two effects, upon irritability and upon duration of total refractoriness, appear to be separable, for though the absolutely refractory stage was prolonged under this procedure, when the nerve could respond at all

[3] Lillie (1925) states (p. 494), "It seems probable that the difference between the absolute and the relative refractory periods in living tissues is an expression of the difference in the character of the transmission in the two periods, this being decremental in the earlier sub-period and complete in the later, as in the two sub-periods of the passive iron model."

It is impossible to tell whether this analogy holds, for the nerve's absolutely refractory period is over in general before the nerve has ceased to respond (electrically), thus the second response, in the absolutely refractory phase, is fused with the first, if there can be considered to be a second response at all, and does not conduct separately, even decrementally. The first *separate* response is conducted indefinitely, as determined by observations at the site of stimulation and at a distance.

at the end of this period the threshold was relatively low. Furthermore, if the current persisted still longer, after refractoriness to an induction shock was recovered from, the irritability either decreased with time or increased, depending on the strength of current and the condition of the nerve; and in the latter event, the nerve could respond again to the constant current.

Secondly, the procedure of the previous experiment was modified as follows. The test induction shock timed just after the end of the absolutely refractory stage of an excitation was made strongly supermaximal (fig. 6 *A*, at *f*). A second shock (*a–c*) entered through the same electrodes, timed earlier, within the absolutely refractory state of the first excitation. If this second shock was made cathodal, *c*, the small action wave, *f'*, from the test shock became still smaller, *c'*, if anodal, *a*, it became larger, *a'*, than without the preceding second shock, though the shock *f* remained supermaximal for all cases.

If these two shocks were sent into otherwise unstimulated nerve, and if the earlier failed to stimulate, its effect would only sum with the later one. Its effect in the refractory phase was probably not a polarization effect upon amplitude or irritability directly. It was rather an indirect effect, upon the duration of the absolutely refractory phase, which brought the subsequent effective stimulus earlier or later in the relatively refractory phase.

If, thirdly, in place of the shock applied during the refractory phase, a constant current was applied, the case was analogous (fig. 6 B). An action potential was initiated by an induction shock, then a constant current of adequate strength was started late in the absolutely refractory phase *b e*, at *a*, and continued until after the resultant second response, *a'*. The first action wave occurred at a time determined by the shock, the second at a time determined both by the refractoriness left by the first process, and by the strength of the constant current (utilization period). Now without moving the position of the induction shock on the time line of the oscillograph, nor changing the strength of the constant current, the beginning of the constant current was moved earlier in the absolutely refractory period, of the first process, *a–c*, again allowing it to flow until a second process resulted. The observations were made at the common cathode of induction shock and constant current. As the constant current began *earlier*, the second process initiated by it moved *later*, *c'*, due only to a greater duration of current flow *during the absolutely refractory period*. Obviously the absolutely refractory state was prolonged due to this factor, unless the irritability during the relatively refractory phase had been reduced; presumably the constant current started to stimulate later, at a time when it began to flow earlier. A still stronger current would prevent a second response altogether. In this case, the refractory state would

seem to be prolonged indefinitely. Further, a very strong constant current stimulus, which is allowed to flow during the resultant excitation, will suppress even this first excitation almost, if not quite, to extinction under the cathode (Bishop, Erlanger and Gasser, 1926a). whereupon the action wave rises again at a distance from the cathode due to spread of stimulating current along the nerve. In all three of the above experiments, the absolutely refractory period appears to be prolonged by cathodal polarization applied *only during this refractory period;* that is, this result does not depend upon long-continued polarization previous to excitation. During the relatively refractory period, cathodal polarization seems to produce lowering or raising of threshold depending on its intensity, as it does in the period preceding excitation.

DISCUSSION. A summary of our own and previous results on the effects of polarization and temperature appears in table 4. A distinction seems immediately possible between the effects of temperature and polarization, in that reduction of temperature appears to reduce in intensity or prolong

TABLE 4

Summary of effects of temperature and polarization on phenomena in nerve activity

+ indicates a numerical increase in value of the designated phenomenon.

EFFECT OF	AMPLITUDE	DEMARCATION POTENTIAL	RATE OF CONDUCTION	DURATION ABSOLUTELY REFRACTORY PERIOD	THRESHOLD	TIME TO MAXIMUM	FALLING PHASE
Temperature increase	±	+	+	−	−	−	−
Anodal polarization ..	+	+	−	−	+	+	+

in time all the observable functions of the nerve, while the effects of polarization are opposite for different functions. Each set of data serves to differentiate the nerve's function into certain components, by its differential effect upon potential changes; e.g., upon duration of rising and falling phases of potential. When the two sets of data are compared, this differentiation is more apparent. For while cooling and cathodal polarization, for instance, act alike in prolonging the refractory state and reducing the amplitude of potential, they act differentially upon duration, irritability and conduction rate. Polarization, in other words, accentuates certain phases of activity and depresses others.

By the concept of polarization used here, we mean to designate not merely the passive concentration of ions at an inert membrane, analogous to the concentration of electrons in a condenser plate. Nerve is certainly not such a system. Rather we have in mind the altered state of reactivity induced, under a potential change, in active or potentially active substances which may be at or near a polarizable surface. Polarization of

such a surface bounding a reactive medium whose ions carry the current will obviously result in an altered equilibrium at the surface, of any reaction into which such ions may enter, due to concentration of the ions at the surface and to the potential drop there. Conversely, any reaction involving the ions producing a state of polarization will alter its potential. Since nerve maintains a potential by its own activity, at its own surface, any external alteration of this potential may be expected to alter the reactions of the nerve until a new state of equilibrium is attained. The nerve "responds" to polarization perhaps in much the same way as it responds to stimulation, without a sudden change, however, that appears as an excitation.

If the rise and the fall of potential as a response to stimulation are signs of distinct processes, which, however they may be related, may be affected differentially, then the resting or unexcited condition of nerve might be looked upon as a condition of equilibrium between them, which may be affected by operation upon either process. The action potential would then be the external sign of the initiation, or perhaps the acceleration, of first one of these processes and then the other, while polarization may be looked upon as a more or less persistent shift in the equilibrium condition. The fact that nerve with excitation gives off an increased amount of CO_2 is consistent with the idea that excitation may be only an accentuation of some phase of its normal resting activity. The fact that it maintains its own polarization during rest may be an expression of the same function that causes restoration of potential after excitation, or after polarization.

If we may further look upon the refractory state as the absence, relative or complete, of some material necessary for excitation, and stimulation as the initiation or acceleration of a reaction in this material when present, it will serve to correlate the different effects of polarization in the unexcited and in refractory nerve. We may assume for this argument that negative potential at the nerve surface tends to cause this reaction to take place, and might lower the threshold by summing its effect with a superposed stimulus. But it might also tend to hinder or prevent the return of irritability after excitation by maintaining a low rate of reaction in the nerve, as the reactive material was produced again. If the nerve were reacting at a low rate, but constantly, under a cathodal polarization, it would be able to react less violently when specifically stimulated. The external potential sign of this steady induced reaction would be masked by the applied negative potential itself. The lowered amplitude of cathodally polarized nerve would be due to the decreased amount of material available for the reaction, together with the cathodal effect on some process of potential restoration, possibly disposal of the first reaction products. The reverse of this hypothesis would apply to anodal effects.

The foregoing is not proposed as an adequate analysis of nerve function,

and many gaps will be apparent for the filling of which the data offer no clue. As an hypothesis according to which the data can be provisionally correlated it may serve a temporary purpose. It represents an attempt to get away from a too naïve expression of the so-called "membrane theory" which is often made to imply that a concentration change in salt ions merely, at or across a membrane, with a concomitant change in electrical or ionic permeability of the membrane, is adequate to account for vital phenomena in nerve.

SUMMARY

The effects of polarization on the activity of nerve have been studied by analyzing the changes in amplitude, irritability and conduction rate, in duration of the rising and falling phases of excitation potential, and in duration of the refractory state, with special attention to the activity near the stimulating cathode, where all the axon waves are in approximately the same phase.

1. Decrease of amplitude under the cathode, increase under the anode of the polarizing current take place with both directions of conduction, as previous workers have reported, and this occurs as well under the stimulating cathode, if the polarizing current is not too strong. The effect is thus not due to a differential effect on different fibers, which might cause differences of amplitude by separation out or recombination of the components of the process as recorded from the nerve trunk at a distance from the point of stimulation. With strong currents which reduce the amplitude under the anode the amplitude at either side of the anodal area may still be increased.

2. Increase of irritability under the cathode, decrease under the anode, of a polarizing current, take place as previously reported, but observations at the site of stimulation and after conduction indicate that the action wave, once started, in a polarized area, may be suppressed by the polarization, and thus give an erroneous picture, after conduction, of the irritability at the point of stimulation.

3. Conduction rate in a polarized area, as measured by short segments of the nerve, and in the fastest fibers, tends in general to be slowed under the anode and increased slightly, if at all, under the cathode. In general the wave travels faster as it passes from a region of high amplitude to one of low amplitude, and faster toward a region of low threshold, and vice versa. Changes in rate are consistent with the notion that the speed of conduction is a function of the intensity of the process in a given section of nerve, *as measured by its action potential*, and of the threshold of the adjacent region, *as measured by the strength of a shock necessary to excite it.* Thus, for instance, the rate increases as the wave approaches a cathodal region of low threshold from the anodal region of high amplitude, even to

above the normal rate for unpolarized nerve, and decreases beyond the cathode as the process works "up hill" again into normal nerve, from a region of lower amplitude to one of higher threshold. The apparent decrement or increment can be assigned to a physiological gradient induced in the nerve by the polarizing current.

4. Changes of temperature affect the rising phase of potential (time to maximum) less than they do the falling phase, suggesting that separate reactions in the nerve may underlie excitation and restoration or recovery. Decrease of temperature prolongs the period of recovery, and vice versa. Polarization phenomena at one temperature are similar to those at others being superimposed upon the results of temperature change.

5. Anodal polarization reduces the absolutely refractory period slightly, cathodal polarization increases it considerably, thus producing the direct opposite to the effect upon recovery, and upon threshold. Lowering of the temperature acts like cathodal polarization in lengthening the refractory period, acts in the opposite manner to cathodal polarization in prolonging the recovery of the action potential. A differentiation between refractoriness and recovery of potential is therefore indicated. The apparent discrepancy between cathodal decrease of threshold and increase of refractory period (which discrepancy disappears with strong polarizing currents) is considered in the discussion.

Polarization in nerve may be interpreted as resulting not merely in a physical potential change, but as comprising an alteration in any fundamental processes taking place at the nerve interfaces acting as electrode surfaces. With increase or decrease of the degree of polarization normally maintained here by the nerve's own activity (demarcation potential) the level of this resting activity, i.e., the dynamic equilibrium of the nerve's chemical reactions, should change correspondingly. In this altered condition its reaction to a stimulus may be looked upon as a typical "all-or-none" reaction, under new equilibrium conditions.

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THE ABSORPTION OF BILE PIGMENT FROM THE INTESTINE

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During the course of our experiments to determine the sites of origin of bile pigment (Mann, Sheard, Bollman and Baldes, 1925), we have made determinations of the bilirubin content of the blood of the mesenteric veins. With the spectrophotometric method of determination of bilirubin in the blood we have demonstrated that blood simultaneously withdrawn from different arteries of the body contains identical amounts of bilirubin. The bilirubin content of blood from venous sources taken at the same time is greater than or identical with that of arterial blood, depending on the area drained by the veins. The splenic, femoral, axillary, jugular and azygos veins were found to contain blood with greater concentrations of bilirubin than arterial blood. During the early development of obstructive jaundice more bile pigment was found in the blood of the hepatic veins than was present in the blood going to the liver in the hepatic artery and portal vein (Mann, Sheard, Bollman and Baldes, 1926). The extension of these findings indicated that bilirubin was added to the blood in the bone marrow, spleen and liver. With the same method we were unable to detect any increase in the bilirubin content of the blood returning from the kidney, the intestine, skeletal muscle or from the entire leg after removal of the bone from that extremity (Mann, Sheard and Bollman, 1926).

Since the spectrophotometric method is accurate and sensitive enough to detect the addition of bilirubin to the blood in the bone marrow, spleen and liver, it would appear that only inconsequential differences in bilirubin concentration would exist in solutions which appear identical by this method. It was therefore rather interesting to note that in none of our experiments did we detect any more bile pigment in the blood of the mesenteric veins or the portal vein after splenectomy than was present in the blood of the mesenteric artery. It would appear that no bile pigment was absorbed from the intestine under the conditions of our experiments. Since this observation was made on a number of animals at different periods following the ingestion of food and under con-

ditions which were not unphysiologic, more importance could be attached to it. We have also observed that the jaundice which follows hepatectomy is not diminished by removal of the intestines at the time the liver is removed.

In opposition to these indications of the absence of absorption of bilirubin from the intestine, we have often observed the rapid disappearance of bile from the small intestine of animals following complete removal of the liver (Mann, Bollman and Magath, 1924). Within two hours after dehepatization the contents of the small intestine assume the clay color characteristic of them following complete biliary obstruction. This finding might be interpreted as evidence of reabsorption of bile pigment, although the factors of peristalsis and bacterial decomposition need also be considered. In view of the findings and especially in view of the conflicting evidence of others regarding the entero-hepatic circulation of bilirubin, a more extensive study of this problem was considered worth while.

LITERATURE CONCERNING INTESTINAL ABSORPTION OF BILE PIGMENT. Previous studies of this subject have been reviewed by Whipple (1922) and by Broun, McMaster and Rous (1923) so that only a brief abstract of the experimental evidence regarding intestinal absorption of bile pigment may be mentioned here. The main evidence against the view that bilirubin is reabsorbed from the gastro-intestinal tract may be summarized in the experiments of Hooper and Whipple (1917). They studied the effect of bile feeding on the secretion of bile in animals with biliary fistula and were unable to detect any increased excretion of bile pigment after administration of large amounts of bile. After determining the rate of secretion of bile pigment, they administered from 100 to 300 cc. of fresh bile by stomach tube and continued the determinations of the rate of secretion of bile pigment for six hours, and in some cases for fifteen hours afterward. Since no increase in the secretion of pigment occurred, they concluded that no intestinal absorption of bile pigment had occurred.

The evidence indicating that bile pigments may be reabsorbed from the intestine may be summarized in the work of Broun, McMaster and Rous. They contended that the experiments of Hooper and Whipple were not extended over a sufficient period of time, and accordingly made twenty-four-hour determinations of the bile pigment excreted in bile-fistula animals. They found that after feeding sheep's bile containing cholohematin, this pigment could be identified by its characteristic spectrum in the bile secreted by the bile-fistula dogs. In addition to this evidence of absorption of a pigment of sheep's bile, they also found that the secretion of bilirubin could be increased by feeding bile to animals with biliary fistulas. In one animal they estimate that 42 per cent of the bilirubin administered in the daily feedings of 400 cc. of bile was recovered in the bile secreted by the liver. Other animals, however, did not

show great increases and, in some, only questionable increases were obtained after bile had been fed. They attributed the lack of absorption of bilirubin in these animals to the fact that gastro-intestinal disturbances frequently followed the administration of large amounts of bile. They reviewed their positive results and pointed out that the increased elimination of bilirubin might have been due to increased destruction of blood attendant upon the absorption of bile salts. Since no proof of this increased destruction could be established, and in view of the demonstrated absorption of other pigments, they concluded that bilirubin was absorbed from the intestine.

EXPERIMENTAL PROCEDURE. The spectrophotometric method for determination of bilirubin in the blood has been described in detail (Sheard, Baldes, Mann and Bollman, 1926). The essential features of this method involve the preparation of an optically clear solution of the bilirubin of plasma and the subsequent determination of the amount of light of specific wave-length absorbed by this solution. The logarithms of the percentages of light specifically absorbed for any specified wave-length by similar solutions are directly proportional to the concentrations of the solutions, and calculations depend on the dilutions involved in preparation of the solution for analysis.

The surgical procedures necessary for this study were performed with aseptic technic, and general anesthesia was produced with ether. The uniformity of the anesthesia was maintained by the use of tracheal intubation and administration of ether vapor from a Wolf bottle of ether over which a uniform amount of air could be admitted. In a number of cases only local anesthesia was employed. This was obtained by infiltration of the zone of operation with 2 per cent novocain solution.

In one group of experiments comparisons were made of the bilirubin content of blood simultaneously withdrawn from the mesenteric veins, or the portal vein after exclusion of the splenic blood, and that from arterial sources. Either general or local anesthesia was employed and the blood specimens were obtained from animals at different periods of digestion. As a routine the animals were fasted for from sixteen to eighteen hours previously and specimens from the fasting animal were obtained at the end of this time. Other animals were given a generous feeding of a mixed diet of meat, bread, milk and syrup, and blood specimens obtained at various intervals from two to six hours after feeding. Other animals were given a feeding of cream and egg-yolk and specimens were then obtained at similar periods after feeding.

In another group of experiments fresh bile from the gall bladder of dogs was injected, in amounts from 100 to 200 cc., into the duodenum, jejunum and ileum. Comparisons were subsequently made of the bilirubin con-

tent of the blood from the mesenteric veins and arterial blood, at periods of from thirty minutes to three hours after administration of the bile.

In another group of experiments the rate of developing bilirubinemia following ligation of the common bile duct and extirpation of the gall-bladder was determined. The subsequent bilirubinemia was determined at intervals for six hours after the injection of 100 to 200 cc. of fresh bile from the gall bladders of dogs into the duodenum, jejunum and ileum. Any intestinal absorption of bilirubin should increase the rate of developing bilirubinemia since excretion of bilirubin was prevented by the ligation of the common bile duct.

The control experiments of this study constitute the largest group of this investigation, and when it was possible these were performed at the same time and in the same animals as were used for the other experiments. The demonstration of the increased bilirubin content of the blood of the femoral vein over that of the femoral artery indicated that the methods employed were extremely sensitive and that the experimental conditions were not unphysiological. The sensitivity of the method was also demonstrated by the greatly increased concentration of bilirubin of the blood which was obtained following the intravenous injection of minute amounts of bile. The absorption of sugar, fat and rose bengal from the intestine was also investigated.

Results. The results of these experiments were uniformly negative in that no absorption of bilirubin from the intestine could be demonstrated. The blood of the mesenteric veins was always found to contain the same amount of bile pigment as the arterial blood. Alterations in diet or variations of the time following feeding was without effect on these findings. At the same time, however, it was shown that absorption of substances other than bilirubin had occurred under the conditions of the experiment. Hyperglycemia had occurred after glucose feeding and lipemia was present after the feeding of fat. That bilirubin was being formed, however, was easily demonstrated in these animals by the finding of more bilirubin in the blood of the femoral vein than was present in the femoral artery.

The enteral administration of bile to these animals did not alter the amount of bilirubin found in the mesenteric veins, the blood of which contained only the same amount of bilirubin as arterial blood. Since determinations were made at intervals up to three hours after as much as 200 cc. of gall bladder bile had been disseminated throughout the entire length of the small bowel, it would appear that no bilirubin was absorbed at this time. At the same time, however, it was demonstrated that fat and glucose administered in the same way could be detected in the blood of the animal. Rose bengal mixed with the bile placed in the intestine could not only be detected in the blood of the animal but also it could be

demonstrated that the dye was being absorbed by the blood passing through the intestine since greater concentrations of the dye were present in the blood of the mesenteric vein.

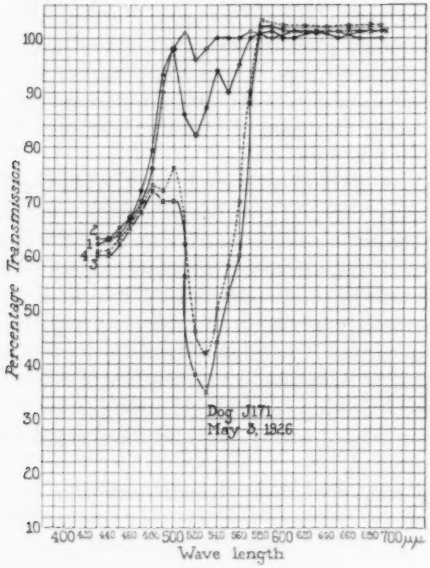

Fig. 1. Curves showing the percentage of light transmission at various wave lengths for equal dilutions of alcoholic extracts of plasma. Curve 1 was obtained from blood withdrawn from the mesenteric vein two hours after the injection of 200 cc. of bile from gall bladders of normal dogs directly into the lumen of the duodenum, jejunum and ileum and thirty minutes after the injection of 100 cc. of 10 per cent glucose and 250 mgm. of rose bengal into the intestine. Curve 2 was obtained from blood of the mesenteric artery withdrawn at the same time. Curves 3 and 4 were obtained from the blood of the mesenteric vein and mesenteric artery respectively, one hour later. The zone of lessened light transmission due to bile pigment (430$\mu\mu$ to 490 $\mu\mu$) shows no difference between the bilirubin content of the mesenteric vein and artery. On the other hand the zone of lessened transmission due to rose bengal (510 $\mu\mu$ to 570 $\mu\mu$) is definitely greater in curves 1 and 3 than in 2 and 4 respectively, because of the greater concentration of rose bengal in the mesenteric vein. The blood sugar content of this animal increased from 185 mgm. per cent to 252 mgm. per cent, thirty minutes after the instillation of glucose into the intestine and to 332 mgm. per cent one hour and thirty minutes after.

The course of developing bilirubinemia was not altered by the injection of 100 to 200 cc. of gall bladder bile into the intestine of animals in which the common bile duct had been occluded and the gall bladder extirpated. These animals continued to become jaundiced at exactly the same rate

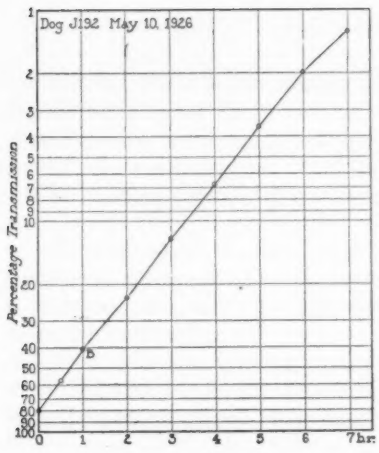

Fig. 2

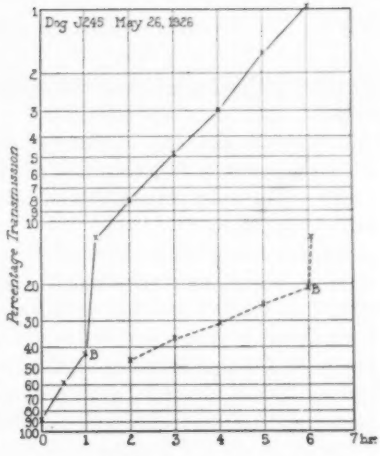

Fig. 3

Fig. 2. Curve showing the increasing bilirubin content of the blood following ligation of the common bile duct and extirpation of the gall bladder. At *B*, 250 cc. of gall bladder bile from normal dogs were injected into the lumen of the duodenum, jejunum and ileum. The bilirubin content of the blood continued to increase at its original rate and was not affected by the addition of bilirubin to the intestine. This curve is similar to those obtained following ligation of the common bile duct and extirpation of the gall bladder without the injection of bile.

Fig. 3. Curve showing the increasing bilirubin content of the blood following ligation of the common bile duct and extirpation of the gall bladder. At *B*, 1 cc. of gall bladder bile from normal dogs was injected intravenously. Immediately after the withdrawal of the next blood specimen fifteen minutes later, 250 cc. of the same bile were injected into the duodenum, jejunum and ileum. Following this the addition of bile pigment to the blood continued at its original rate until four and three-fourths hours later when the rate of addition of bile pigment was definitely increased by the repetition of the injection of 1 cc. of bile intravenously. The solid line was made from readings obtained from extracts of plasma with the usual dilution (1:4) and the broken line was made from readings with further dilution of this extract by the addition of two volumes of alcohol. Note that there is no evidence of the absorption of bile pigment from the intestine but that the intravenous injection of $\frac{1}{250}$ of the amount placed in the intestine produced a definite break in the curve as indicated at the points marked *B*.

Note. The curves of figures 2 and 3 were made by plotting the values obtained for the wave length 430 $\mu\mu$ in the curve of the light transmission for alcoholic extracts of equal dilution of plasma from each blood specimen obtained. According to the laws of Lambert and Beer, the concentration of the pigment in solutions is inversely proportional to the logarithm of the intensity of the light transmitted at any specified wave length which is specifically absorbed by the substance in solution. For this reason the plotting of the actual readings obtained for the transmission of light at 430 $\mu\mu$ on an inverted logarithmic scale gives the relative concentrations of bile pigment in the different specimens as the linear distances of these ordinates. With the time of withdrawal of the blood specimens as abscissas the straight line obtained in these curves indicates that bile pigment was accumulating in the blood at a uniform rate.

as similar animals without the enteral administration of bile, and no increased rate could be determined for as long as six hours after enteral administration of bile. Intravenous injection of less than one-hundredth of the amount of bile that had been placed in the intestine was sufficient to give a measurable amount of increase of the bilirubin content of the blood (figs. 1 to 3).

Discussion. The results of these experiments indicate quite clearly that no intestinal absorption of bilirubin occurred under the conditions which we employed. The principal objection which can be raised to this method of experimentation seems to be that the amount of bilirubin available for reabsorption in the fasting or actively digesting animal is too small to be detected even if absorption did take place. It would seem, however, that a method sufficiently sensitive to detect the formation of bilirubin in one of the many sites of bilirubin formation of the body, would be sufficiently sensitive to detect the reabsorption of any appreciable amount of the bile pigment secreted from the entire body.

In the experiments involving enteral administration of bile, it might appear that sufficient time had not been allowed for the absorption of this material. It should be recalled that some time was gained over feeding experiments by the direct instillation of the bile into the intestine, and interposition of the liver to excrete the absorbed pigment was not necessary. It was also demonstrated that rose bengal, a dye excreted specifically by the liver, was readily absorbed from the intestines under the conditions of these experiments.

While the results of these experiments seem to indicate definitely that no bile pigment was absorbed from the intestine, they offer no explanation for the findings of Broun, McMaster and Rous which did show absorption of bile pigment from the intestine. It seems quite reasonable to correlate these two divergent views with the opinion that bile pigment may be absorbed from the intestine under certain conditions. The question then arising is whether the intestinal absorption of bile pigment is a normal process or one that occurs only under abnormal conditions. Several of the animals with bile fistula used by Broun, McMaster and Rous failed to show evidence of the absorption of bile pigment from the intestine. They attributed the absence of absorption to the diarrhea produced by the feeding of bile, considering that the bile pigment passed through the intestine too rapidly to allow time for absorption. The animal which they found had the greatest intestinal absorption of bilirubin also had an intestinal obstruction which delayed the passage of the bile through the intestine. It may be that this obstruction also produced changes in the permeability of the intestine. Our experiments were performed with animals which had not been subjected to previous operation, and although our data were obtained soon after some operative measures were

taken, we found no evidence that the intestine was abnormal when the experiments were performed.

SUMMARY

The bilirubin content of the blood of the mesenteric veins is essentially the same as the bilirubin content of arterial blood. This is equally true whether the specimens are obtained from a fasting animal or from animals at different periods of digestion or whether large amounts of bile have been previously introduced into the intestines. The methods employed in this study are sufficiently sensitive to detect the addition of bilirubin to the blood as it passes through any of the sites of bilirubin formation, and the direct absorption of rose bengal from the intestine is easily demonstrated by the finding of much more of this dye in the blood of the mesenteric veins than is contained in the arterial blood. Large amounts of fresh gall bladder bile administered directly into the intestine are without effect on the rate of developing jaundice following ligation of the common bile duct and extirpation of the gall bladder. It may be definitely stated that no intestinal absorption of bilirubin occurs under the conditions of these experiments.

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STUDIES IN AVIAN DIABETES AND GLYCOSURIA

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Attempted pancreatectomy of birds antedates v. Mehring and Minkowski's classical discovery of the experimental pancreatic diabetes in dogs (1889). Claude Bernard, after unsuccessful attempts at complete pancreatectomy in the dog, is apparently the first to have performed the operation on pigeons and ducks (1877). The birds rapidly developed symptoms of "marasmus" and died in from ten to twelve days. Langendorff (1879) ligated the pancreatic ducts in the pigeon. The animals lost weight very rapidly and died in from six to twelve days, due apparently to inanition. He emphasized the point that glycosuria did not develop in these animals even after the ingestion of large amounts of sugar. He observed glycosuria in the hawk after pancreatectomy. Langendorff maintained that the acini of the pancreas were degenerated, and the liver was devoid of glycogen. Minkowski (1893) repeated the experiments of Bernard and removed by careful dissection the pancreas in pigeons and ducks. The three pigeons operated on died on the third or fourth day, and the two ducks operated on were killed on the seventh and eighteenth days. The lack of the external pancreatic secretion was evident in these animals but glycosuria did not occur, even after the administration of 15 grams of sugar. Weintraub (1894) carried out similar experiments on a larger scale. He operated on nineteen ducks, removing the pancreas with the entire duodenum and suturing the ileum to the stomach, because he had observed that the removal of the pancreas alone causes necrosis of the duodenum. He emphasizes the point that one should not injure the ductus choledochus during the operation. Weintraub observed glycosuria in four cases and no glycosuria in fifteen. He did not make histological examination. Some of his animals survived only a few days, others one or two weeks and a few a "long time." He maintained that the administration of dextrose to normal birds failed to produce glycosuria whereas large amounts of saccharose did. Kausch (1896) continued the experiments of Weintraub. He removed the pancreas of a large number of geese and ducks, essentially according to the method of Weintraub. Most of his

ducks died from one to two days after operation; one lived fifty days. He observed, contrary to the findings of Weintraub, a distinct hyperglycemia in ducks, which gradually disappeared after twenty-four hours. Seven showed no increase in blood sugar, twenty-eight a moderate increase (0.2 to 0.3), and forty-eight a marked increase with the blood sugar as high as 7 mgm. per cubic centimeter. No glycosuria was observed. He explains this phenomenon by the assumption of a relative impermeability of the avian kidney for glucose; but he suggests another possibility, namely, that the avian kidney may be as permeable for glucose as is the mammalian kidney, but that the sugar is consumed by the kidney instead of being excreted. In a few instances, however, he did observe sugar in the urine of the operated birds. Twenty-four hours after pancreatic extirpation the liver was practically glycogen-free. Even the ingestion of excessive amounts of foodstuffs did not cause storing of glycogen in the liver. In geese Kausch found that after pancreatectomy the blood sugar went up slightly during the first twenty-four hours, after which it went up for a time slowly, then later more rapidly, reaching a maximum in one case on the twenty-seventh day, and in others on the tenth and eleventh days. These authors did not work on the fowl. The only investigation on the fowl of which we are aware was carried out by Giaja (1912). He determined the normal blood sugar of the fowl according to the Mohr-Bertrand method, and found this varies from 1.75 mgm. to 2.4 mgm. per cubic centimeter of blood. After the excision of the pancreas he found that the blood sugar of the operated fowls varies from 1.70 mgm. to 2.6 mgm. per cubic centimeter, and considers this an evidence of hyperglycemia. How long his fowls lived after pancreatectomy is not stated beyond the fact that the blood sugar determinations were made within thirty hours from the time of operation. He did not find glycosuria in the operated fowl. Finally, he found that the intravenous injection of 40 grams of glucose in a normal fowl of 1.6 kgm. body weight caused marked glycosuria.

EXPERIMENTAL. In view of the conflicting literature we decided to carry on our experiments on a single species, the domestic fowl (gallus domesticus). We used a large number of white leghorn and Rhode Island reds. Our experiments can be classified into two groups. The first one was to produce experimental glycosuria in the fowl, and the other naturally connected with the former, to observe the effects of the complete removal of the pancreas. At first we carried out a number of blood sugar determinations in the normal fowl (cocks and hens) by the Folin-Wu colorimetric method. We found, as illustrated in table 1, a great variation of the normal blood sugar values in both sexes. There seems to be no essential difference between the normal blood sugar level in cocks and in hens. In a male, for instance, the normal blood sugar varied from

2 to 4.39 mgm. per cubic centimeter, and in a female it varied from 2.01 to 3.14 mgm. per cubic centimeter. Because of this extreme variation of the normal blood sugar values we suspected that the blood sugar depends on the state of excitement during the drawing of the blood from the vein of the wing. Therefore, we tried to excite the chicken purposely by tying a strong cord around the legs and pulling it for a period of fifteen minutes. We found however, that the blood sugar was only 2.1 mgm.

TABLE 1

Normal blood sugar of fowl on different days (no. 1 male; no. 2, female)

ANIMAL NUMBER	BLOOD SUGAR	ANIMAL NUMBER	BLOOD SUGAR
	mgm. per cc.		*mgm. per cc.*
1	2.0	2	3.14
	2.10		3.06
	2.9		3.03
	4.0		2.01
	3.0		2.90
	4.39		2.65
	4.2		
	2.85		

TABLE 2

The effects of subcutaneous injections of dextrose in the fowl

ANIMAL NUMBER	NORMAL BLOOD SUGAR	DEXTROSE INJECTED	TIME ELAPSING BETWEEN INJECTION AND TESTS	BLOOD SUGAR AFTER INJECTION	FEHLING TEST OF URINE
	mgm. per cc.	*grams*	*minutes*	*mgm. per cc.*	
1	2.0	20.0	30	5.0	+
2	3.3	10.0	30	4.0	+
3	4.0	10.0	45	5.5	+
4		5.0	60	2.85	−
5		5.0	60	4.59	−
6	3.6	2.5	30	2.5	−
7	3.0	2.5	30	4.4	−
8	2.66	5.0	60	2.75	
9	2.1	5.0	60	3.07	−

per cubic centimeter of blood, exactly the same value which was obtained on the the same chicken without previous excitement. It is needless to say that in this chicken there was no trace of sugar in the urine; so we could not find, at least in this specimen, any evidence of emotional hyperglycemia or glycosuria.

To test the conflicting views about the relative impermeability of the kidneys for glucose we carried out a number of experiments, applying the factors which produce glycosuria in mammals. At first we injected from

2.5 to 20 grams of glucose subcutaneously into chickens weighing on the average 1.5 kgm. The injection of 2.5 grams of glucose did not produce any appreciable rise in the blood sugar. Neither did it cause glycosuria. The injection of 5 grams of glucose was also without any effect on the blood sugar, and was insufficient to produce glycosuria. The administration of from 10 to 20 grams of glucose produced invariably an appreciable hyperglycemia and a strongly positive Fehling test of the urine (table 2). One to three cubic centimeters of epinephrin solution (1:1000, Parke, Davis & Co.) was injected hypodermically into various fowls. This caused a rise in the blood sugar and an abundant excretion of sugar in the

TABLE 3

The effects of subcutaneous injections of epinephrin in the fowl

ANIMAL NUMBER	NORMAL BLOOD SUGAR	ADRENALIN INJECTED	TIME ELAPSING BETWEEN INJECTION AND TEST	BLOOD SUGAR AFTER INJECTION	FEHLING TEST OF URINE
	mgm. per cc.	*cc.*	*minutes*	*mgm. per cc.*	
1	4.39	2	75	4.7	+
2	2.8	2	75	3.3	+
3	4.2	3	80	4.4	+
4	2.35	1	82	2.9	+
5	2.63	1	83	4.0	+

TABLE 4

The effects of subcutaneous injections of phlorhizin in the urine of the fowl with anal fistula

ANIMAL NUMBER	PHLORHIZIN INJECTED	URINE COLLECTED IN ONE HOUR	FEHLING TEST	ALBUMIN	ACETONE	ACIDITY
	gram	*cc.*				
1	0.8	20	+	−	−	+
2	0.8	15	+	−	−	+
3	0.8	10	+	−	−	+

urine. The optimal time for determining the blood and urine sugar after epinephrin injections is between seventy-five and eighty minutes (see table 3). Finally, we injected 0.08 gram of phlorhizin in alcoholic solutions subcutaneously in various fowls. In about one hour after the injection we were always able to detect an abundance of sugar in the urine, though tests for acetone and albumin were negative (table 4). It may be mentioned in this connection that we collected the urine by establishing an anus preternaturalis (colostomy). In other words, we sewed a loop of the rectum to the abdominal skin, and closed up very carefully the remaining portion of the large bowel in order to prevent peritonitis. In such a way we were able to collect clear urine through the cloaca. La-

ter on we found that this procedure is unnecessary and that any mechanical or chemical stimulation of the inner surface of the anus will result in the emission of clear urine.

The next step in our experimentation was to remove the entire pancreas. Giaja did not describe his method of extirpation of the pancreas in the fowl, so we could rely only on the experience of Kausch and Weintraub. After many unsuccessful attempts to remove the whole pancreas this surgical problem was evolved by one of us (Ivy), and we were able by his method to keep the birds alive. The fowl was anesthetized with ether and placed on a special board so constructed that the wings would be held firmly, the legs abducted, and the body inclined with the head downward at an angle of fifteen degrees. This position facilitated the operative pro-

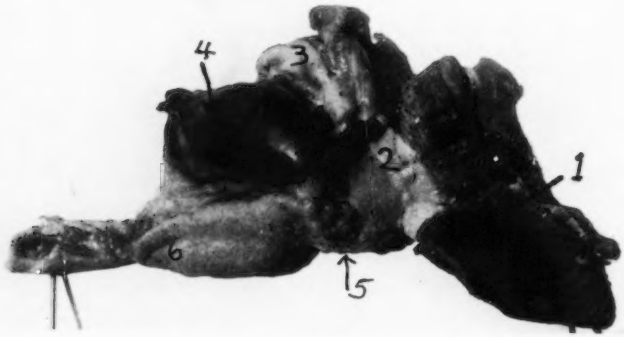

Fig. 1. Showing specimen of end-to-end anastomosis in a chicken killed 44 days after total pancreatectomy and duodenectomy. *1*, stomach; *2*, pyloric sphincter; *3*, liver; *4*, gall bladder; *5*, end-to-end anastomosis; *6*, small intestine.

cedure. The abdomen was opened under aseptic precautions, and the duodenum and pancreas were delivered to the outside. The small blood vessels in the posterior mesentery of the duodenum were ligated. The duodenum was freed up to the bile ducts and close to the pylorus. At this point small, specially constructed low compression bulldog clamps were applied to the duodenum, one about $\frac{1}{4}$ inch caudally to the pyloric sphincter, the other cephalically to the opening of the bile duct. Sometimes the anatomical location of the pancreas forced us to place the clamp caudally to the bile duct, in which case the bile duct had to be ligated. (This was done in our last chicken, killed 44 days after the operation.) The duodenum was again clamped with mosquito forceps placed about $\frac{1}{2}$ inch away from the bulldog clamps. The duodenum was cut between

TABLE 5

Effects of pancreatectomy in the fowl

ANIMAL NUMBER	TIME	WEIGHT	BLOOD SUGAR	URINE SUGAR	NOTES
		kgm.	*mgm. per cc.*		
1	August 19				Food withheld until after operation
	August 21				Pancreatectomy
	August 24	2.1	5.75	?	
	August 26	2.0	8.80	+	
	August 28	2.0	1.90	−	
	August 31	1.9	2.42	−	
	September 3		2.05	−	
	September 25	1.7		−	
	September 29				Found dead
2	October 22				Food withheld until after operation
	October 24				
	October 27	1.8	6.6	+	Pancreatectomy
	October 29	2.0	4.0	?	
	October 30	1.9	3.3	?	
3	July 13				Food withheld until after operation
	July 15				Pancreatectomy
	July 18			+	
	July 20	1.7	6.95	+	
	July 24	1.6	2.10	−	
	July 27	1.6	2.28	−	
	August 14				Found dead
4	November 18				Food withheld until after operation
	November 20				Pancreatectomy
	November 24		8.9		
	November 25				Found dead
5	March 25				Food withheld
	March 27				Pancreatectomy
	March 31		3.84	+	
	April 1		4.38	+	
	April 8		3.00	+	
	April 17				
	10.15 a.m.		1.40	−	Injected 2 cc. adrenalin intravenously
	11.00		3.00	+	
	12.00 p.m.		3.00	+	

TABLE 5—*Concluded*

ANIMAL NUMBER	TIME	WEIGHT	BLOOD SUGAR	URINE SUGAR	NOTES
		kgm.	*mgm. per cc.*		
5	3.30		3.00	+	
	April 18		1.43		
	April 19		1.40		
	May 10		1.72		Killed. Glycogen found in liver and muscle
6	March 11				Food withheld
	March 13				Pancreatectomy
	March 15		3.18		
	March 16		4.19		
	March 17				Animal dead
7	March 3				Food withheld
	March 5				Pancreatectomy
	March 8		6.18		
	March 9				Animal dead

the forceps and clamp, the exposed mucosa being cleaned with gauze. A ligature was then placed about the large principal blood vessels of the duodenum and pancreas in such a manner that the tip of the pancreas was not included in the ligature. The pedicle was cut, which procedure completely removed the pancreas and adjacent duodenum. The integrity of the gut was then reëstablished by an end to end anastomosis (see fig. 1). This was the most difficult part of the operation because of the friability of the tissues and the difficulty of access. Pulling down on the stomach to facilitate the work was found to be impossible because respiration was thereby prevented. Healing of the end to end anastomosis usually occurred satisfactorily. However, we lost some fowls by peritonitis, through failure of the anastomosis, or as an after-effect of the anesthesia. We consider only three fowls as really successfully operated. The first chicken lived forty days, the second thirty days, and the third one was killed forty-four days after the operation. We found it advisable to starve the fowls one or two days prior to the operation. The general findings on these animals may be summarized as follows. During the first week following the operation the blood sugar rises to an unusually high level. In one animal which lived only for a few days, the blood sugar rose to 13 mgm. per cubic centimeter of blood. When hyperglycemia was present we always found a marked glycosuria. We also observed a polydipsia to such a degree that the crop was distended with water. These animals may be considered to be typically diabetic. After a period of about a week the situation is essentially changed. The blood sugar goes

back to the normal level, never exceeding 3.5 mgm. per cubic centimeter of blood. The sugar disappears entirely from the urine; the crop returns to the normal size; and the animal lives on a normal diet with neither hyperglycemia nor glycosuria. For some time it maintains its weight, but after the third week it shows distinct symptoms of inanition. There are large fat droplets present in the feces. In other words, the animal shows the usual digestive disturbances due to the lack of external pancreatic secretion.

Microscopic examination of the tissue at the site of pancreatectomy revealed no pancreatic tissue. As far as we have been able to learn the chicken does not have accessory pancreatic tissue.

The effect of epinephrin in the diabetic fowl was then studied. We injected 2 cc. of adrenalin (1–1000) hypodermically into a chicken whose pancreas had been removed three weeks previously. At the time of the epinephrin injection blood sugar was unusually low, or 1.4 mgm. per cubic centimeter of blood. The blood sugar rose to 3.0 mgm. and the urine showed a strongly positive Fehling test. The animal was killed forty-four days after the operation. The liver contained glycogen, as determined by a qualitative test. In this connection it may be mentioned that the pancreas of the normal fowl yields insulin as determined by the hypoglycemia test in rabbits.

Discussion. The view of previous authors that the avian kidney is impermeable to glucose is no longer tenable. Phlorhizin, as already mentioned by Minkowski, produced a marked glycosuria; furthermore, parenteral sugar administration produced glycosuria. These experiments show that the kidneys are permeable to glucose. However, the normal blood sugar of the bird may be chemically different from the hypodermically or intravenously administered glucose; and the threshold of the avian kidney may be very high for its own blood sugars. This seems not unlikely in view of the possible existence of alpha, beta and gamma blood glucose. But even this possibility seems remote; for if we inject adrenalin we mobilize the stored glycogen from the liver, the usual source of the blood sugar, and this procedure results in a marked glycosuria; and further, contrary to previous investigators, there is a very marked but temporary glycosuria following a total ablation of the pancreas, which proves that the avian kidney is permeable to the glucose of the blood. The only difference between a mammal and a bird in this respect is the fact that the normal blood sugar level of the bird is usually much higher than that of the mammal; and it is furthermore true that even when the normal blood sugar is 4 mgm. per cubic centimeter of blood, there may be no trace of sugar in the urine.

The most surprising fact revealed by our experiments is the development of the pancreatic diabetes in the chicken, and its disappearance after

seven days. The further fact that the chicken's pancreas yields active insulin, permits two possible explanations of the temporary diabetes. During the first week after pancreatectomy another organ may develop the ability to regulate carbohydrate metabolism. In other words, another organ, probably the liver, may develop the faculty of caring for carbohydrate metabolism in some unknown manner. The second possibility is that insulin production is not solely specific for the pancreas, though the pancreas would appear to be the main producer of this hormone. The avian pancreas being relatively larger than the mammalian may produce and store relative larger quantities of insulin, and the sudden failure of this source may cause the temporary diabetes until compensated for by other tissues with increased insulin production. That there is no true diabetes in the fowl seven to ten days after complete pancreatectomy is shown by the fact that the blood sugar is normal, there is no glycosuria, and the liver contains glycogen and appears normal in structure.

SUMMARY

1. The normal blood sugar of the chicken varies from 1.5 to 4.5 mgm. per cubic centimeter of blood.

2. The hypodermic injection of 10 to 20 grams of glucose results in a marked hyperglycemia and glycosuria.

3. Adrenalin produces hyperglycemia and glycosuria in the normal and depancreatized domestic fowl.

4. Phlorhizin produces glycosuria in the fowl.

5. The total removal of the pancreas in fowls results in typical diabetes for the first 6 to 8 days. After that period the blood sugar returns to normal, the sugar disappears from the urine and the liver stores glycogen. The animals die apparently from digestive disorders and inanition.

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III. MUSCLE HEMOGLOBIN AS A SOURCE OF BILE PIGMENT[1]

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That the *hemoglobin of the blood is the source of all bile pigment* is a statement in accord with current opinion. We believe that the above thesis is mistaken in several particulars but at this time we propose to show that *muscle hemoglobin* also is a source of bile pigment.

There is an extraordinary difference in the volume of experimental work dealing with blood hemoglobin and muscle hemoglobin. Almost every laboratory of medicine, pediatrics, pathology or physiology has contributed one or more papers dealing with blood hemoglobin and its relations to body function. The papers dealing with muscle hemoglobin by contrast are hard to find and not easy to bring into harmony, one with another. Perhaps the chief reason for this striking difference in work done is the fact that the blood hemoglobin is very easy to obtain and analyse but muscle hemoglobin is hard to obtain free from blood hemoglobin and difficult to separate from the muscle proteins.

A method has been described recently (Whipple, 1926) which in our hands gives accurate quantitative figures for dog muscle hemoglobin and the average values of different striated muscles under different conditions were established. The most important single factor in determining the physiological level of hemoglobin storage in striated muscle seems to be functional activity. We were somewhat surprised to find that exercise was more potent than severe anemia—in other words, we find lower muscle hemoglobin values in inactive house dogs with normal blood hemoglobin than in active dogs with one-third normal blood hemoglobin.

It was pointed out (Whipple, 1926) that striated muscles in dogs may comprise 25 to 40 per cent of the entire live body weight. When we realize that under a great variety of conditions the total muscle hemoglobin may range from 10 to 80 grams in relation to the blood hemoglobin repre-

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sented as 100 grams it is obvious that muscle hemoglobin is not insignificant in any phase of hemoglobin metabolism within the body.

Kennedy and Whipple (1926) reported some experiments to indicate that muscle and blood hemoglobins possess many physical properties in common. If these substances are not identical they are so much alike as to be almost indistinguishable. One might suspect that complex substances of this nature might break down or be katabolised in the body in a uniform fashion. We know that much of the hemoglobin freed in the blood stream during body wear and tear will appear in the form of bile pigment. One would suspect therefore that muscle hemoglobin worn out in the striated muscles would appear in part at least in the form of bile pigment. The experiments given below confirm this suspicion and we observe that muscle hemoglobin (freed from blood hemoglobin) introduced into any part of the body or circulating blood will cause the prompt appearance of bile pigment in the urine. It is evident therefore that the body can break down muscle hemoglobin just as it does blood hemoglobin into bile pigments. This may be submitted as additional evidence that these two important hemoglobins are almost if not quite identical.

We know that during long periods of hard physical exercise, as in training, a hunting dog will store a maximum amount of muscle hemoglobin which is a most important reserve against the explosive liberation of energy which these striated muscles may exhibit. We have shown (Whipple, 1926) that anemia and lack of exercise may reduce this high level of muscle hemoglobin and that muscular paralysis (sciatic nerve section) may reduce this level still lower. Yet we do not understand the mechanism of body construction of muscle hemoglobin and we do not know whether the building of muscle hemoglobin is as dependent upon diet factors as is the construction of blood hemoglobin. We have experiments in progress which may answer the last question.

Red cells are worn out in a mechanical sort of way; the cells disintegrate, the stroma presumably is collected by phagocytes and the hemoglobin digested by endothelial cells, to be disposed of as bile pigment or recast into new hemoglobin. Recent experiments of ours soon to be published show that under conditions of severe anemia the dog may conserve for new hemoglobin production as much as 50 to 80 per cent of all introduced hemoglobin,—truly a remarkable conservation under stress.

We may inquire whether the body will conserve its muscle hemoglobin under similar stress and as yet no answer can be given. Moreover the muscle cell does not disintegrate like the red cell. It is more or less a permanent institution and its regeneration after a destructive injury is a difficult if not impossible task for the body. We must assume of necessity that its elements wear out and are replaced without disturbing the

fabric of striated fibres, sheaths and nuclei. The muscle hemoglobin is a very important part of this fabric and its substance must be worn away and used up by physiological activity and by some unknown mechanism replaced to normal. The experiments below indicate that one end product of such wear and tear of muscle hemoglobin may be bile pigment. We hope to find the diet substances which may control the reconstruction of this muscle hemoglobin.

METHODS. The preparation of muscle hemoglobin free from blood hemoglobin was described in a recent paper (1926) and need be reviewed only in brief. The dog under ether anesthesia is freed from blood by simultaneous equivalent bleeding and perfusion with a Ringer's solution containing 5 per cent glucose. The red cell hematocrit by this means is reduced from 50 per cent (normal) to $\frac{1}{2}$ to 3 per cent. Immediately on cessation of heart beats the thorax is opened and viscera removed. The legs and back are then vigorously massaged to eliminate all diluted blood remaining in the vessels. The muscles are dissected out promptly, washed and massaged thoroughly in normal saline and dried. This muscle will be found free of any red corpuscles in the capillaries. The muscle is immediately ground in a meat grinder of ordinary kitchen type, mixed with 3 volumes of distilled water or a 0.4 per cent ammonia solution. This mixture with repeated mixings is kept in an ice box at 4°C. for 20 hours. The dilute ammonia solution extracts much more muscle hemoglobin than does distilled water but there is no apparent difference in the reaction of these solutions within the living animal. At times these solutions are very toxic or even lethal but such experiments are not included in this series.

This red solution is now filtered through gauze and paper, then centrifugalized at high speed for 30 minutes and finally sterilized by passage through a medium Berkefeld filter. This last operation removes a good deal of the muscle hemoglobin but it is a necessity for the preparation of fluids to be injected as indicated. The amount of muscle hemoglobin is measured accurately in each sterile sample. These final solutions are kept in sterile containers in the ice box until used for injection and may be preserved even 3 to 5 days but they are prone to become toxic after 5 to 6 days. Before injection some solutions were made up to 3 per cent dextrose to insure hypertonicity but it is probable that these solutions contain sufficient salts and muscle protein to guarantee against possible hemolysis from injection of the solution. The solutions were given through a small hypodermic needle by gravity and a very slow flow was used, an ordinary injection lasting from 15 to 30 minutes.

Dogs were used in all these experiments in which muscle hemoglobin was injected. They were all in a healthy condition, usually adult females to facilitate catheterization. They were kept in metabolism cages during

the experimental periods. No food was given for 24 hours preceding and following the injections. Urine samples were always examined for bile pigment before the injections as not a few dogs in active health excrete small amounts of bile pigment in the urine. These control readings are not included in the tables as the dogs which showed traces of bile pigment were rejected. At times the muscle hemoglobin solutions are toxic and in a few experiments fatal intoxication developed. The experiments here given showed no clinical intoxication or at the most salivation and depression for two or three hours.

Bile pigments in urine or serum were demonstrated by the familiar Huppert test as described previously (Hooper and Whipple, 1916). The Van den Bergh test was used as control but no (+) readings are recorded where the Huppert test was negative.

EXPERIMENTAL OBSERVATIONS. Our first experiments concerned the pleural cavity into which 200 to 300 cc. of muscle hemoglobin solutions were introduced. After one, two or three days the dog was killed with ether and the pleural contents removed for analysis. We found that in spite of every precaution the needle used for injection would wound slightly the moving visceral pleura causing a little hemorrhage. We found positive bile pigment in many experiments but the possibility of this pigment coming from red cells (hemorrhage) could not be excluded.

We turned therefore to intraperitoneal injection and found that injections could be made here without any bleeding or injury to the peritoneum. For example, dog 25–38 (table 31) 24 hours after the second intraperitoneal injection was killed with ether and examined carefully. The injection was made through the linea alba just below the umbilicus with a small needle. At autopsy the peritoneal cavity was clean and normal—no evidences of hemorrhage. There was a little bit of free fluid in the peritoneum (2 to 4 cc.) which in smears contained a few polymorphonuclear leucocytes and large phagocytes but no bacteria and no red cells.

Table 31 gives the necessary experimental data on a sufficient number of experiments. Other confirmatory experiments have been performed but need not be published. Some anesthetic as indicated was usually given, not because the injections caused any pain, but to insure absolute quiet during the long period of injection to guard against slight injury by the needle point of the visceral peritoneum. It will be seen that most of the bile pigment appears in the night urine which usually contains enough bile pigment to give a strong green color to the final acid alcohol solution. At times a dog will be able to tolerate an injection of 200 mgm. muscle hemoglobin without demonstrable bile pigment appearing in the urine. Many dogs were observed during the second 24 hours after the injection and some show small amounts of bile pigment. There was not sufficiently rapid absorption from the peritoneum to give tests for hemoglobin in

the plasma during the 6 hours following the injection. A time interval of 1 week intervened between the two experiments of dog 25–38.

Intramuscular injection (table 32) gives further evidence that solutions of muscle hemoglobin introduced into the body are promptly broken down into bile pigment and other end products. The first two experiments show abundant bile pigment in the night urine and in the first there was

TABLE 31

Intraperitoneal injection

DOG NUMBER	MUSCLE HEMOGLOBIN H_2O EXTRACT		URINE			WEIGHT	REMARKS
			Bile pigment		Hemoglobin 6 hours		
			6 hours	20 hours			
	cc.	*mgm.*				*kgm.*	
25–59	750	983	++	+	0	20.5	No anesthesia
25–38	250	328	0	+++	0	5.2	Morphia
25–38	250	328	+	+++	0	5.2	Ether anesthesia (killed)
25–64	400	236	+	+++	0	4.5	Ether anesthesia
25–64*	300	423	0	++	0	4.1	Ether anesthesia

Blood plasma clear during 6 hours following injection and plasma bile pigment tests negative.

* Second injection 3 weeks after the first—in this experiment the dilute ammonia muscle hemoglobin extract was used.

TABLE 32

Intramuscular injection

DOG NUMBER	MUSCLE HEMOGLOBIN H_2O EXTRACT		URINE			WEIGHT
			Bile pigment		Hemoglobin 6 hours	
			6 hours	20 hours		
	cc.	*mgm.*				*kgm.*
25–55	500	655	?	+++	++	10.8
25–59	400	524	0	++	0	20.5
25–59	400	236	0	0	0	20.5

Ether anesthesia about 30 minutes during the hypodermic injections into muscles of fore and hind legs and back.

some escape of the muscle hemoglobin in the urine. The size of the dose is one factor which appears to determine whether the muscle hemoglobin will escape in the urine. The last dog shows that a certain amount of muscle hemoglobin can be taken care of in the normal body without escape of bile pigment in the urine. Whether this means total elimination through the liver or conservation for other body use we cannot say at this time.

The muscle hemoglobin solutions in these experiments were given slowly by gravity using a fine hypodermic needle introduced into the deep extensors, adductors, triceps, etc., of both fore and hind legs and the deep erector spinae group on both sides.

It may be objected that muscle puncture of this sort may cause slight hemorrhage which would be responsible for bile pigment in the urine. We controlled this point as follows—a dog (25–39) was given 10 cc. of whole blood taken from its own jugular vein into the deep adductors of the hind legs. In every respect this experiment was carried out like the other intramuscular injections. This blood contained 1850 mgm. of hemoglo-

TABLE 33

Intravenous injection

DOG NUMBER	MUSCLE HEMOGLOBIN NH_3 EXTRACT		URINE			WEIGHT	REMARKS
			Bile pigment		Hemoglobin 6 hours		
			8 hours	20 hours			
	cc.	*mgm.*			*mgm.*	*kgm.*	
25–66	300	423	0	++	93	16.4	Normal dog
21–67	500	705	+	0	161	11.1	Anemic dog
21–67	700	1008	0	+	498	11.1	Anemic dog
21–67	600	846	+	++	676	11.0	Anemic dog
21–23	200	294	+	0	134	17.7	Anemic dog
21–23	335	542	++	0	0	17.6	Anemic dog
21–23	350	566	+	++	77	17.7	Anemic dog
24–25	450	662		++	0	15.2	Anemic dog
24–25	525	850	+	+	291	15.2	Anemic dog
24–25	350	566		0	0	15.4	Anemic dog
24–45	1000	1050	+++	0	++	19.7	Anemic dog
24–45	1000	1100	+	0	720	20.3	Anemic dog
24–45	1000	870	+	0	406	20.9	Anemic dog

Dog 24–45 given water extract of muscle hemoglobin instead of dilute ammonia extract.

bin. The dog showed not a trace of bile pigment in the urine for 48 hours after injection. Evidently prompt coagulation and slow autolysis is responsible for this very slow elimination of this hemoglobin. When one injects a like amount of whole blood into the peritoneal cavity there will be bile pigments excreted in the urine during 24 and 48 hour periods.

Table 33 gives a very small number of the many experiments we have done with intravenous injection of solutions of muscle hemoglobin. We have done most of such injections to study the utilization which the anemic dog can make of injected muscle hemoglobin. These data are presented in this form to show that bile pigments appear promptly in the

urine even in anemic dogs when muscle hemoglobin is given intravenously. There are peculiar fluctuations in the elimination of the muscle hemoglobin and bile pigments in the urine which we cannot explain in the light of present knowledge. We are confident that these solutions by themselves do not cause hemolysis within the circulation. The solutions are given through a small needle by gravity, therefore very slowly, and many solutions were made up to a 3 per cent solution of glucose as further safeguard. Occasionally an anemic dog (see 24–25, table 33) will absorb 500 mgm. of muscle hemoglobin without excretion of any bile pigment in the urine but this is the exception and we cannot as yet explain these peculiar fluctuations.

Discussion. In a recent paper by Rous and Drury (1925) the following statement is made: "The coloring matter responsible for jaundice is derived from blood pigment and, to all appearance, from that alone." This statement is in accord with the general belief and probably represents accurately current opinion among physiologists and physicians alike. Yet the experiments above show that the statement is erroneous as *muscle hemoglobin* must be included with blood pigment as a source of bile pigment which is the coloring matter responsible for jaundice. Who knows the relative speed of breakdown of red cell and muscle hemoglobin? Can any physiologist predict as to this ratio of hemoglobin disintegration in red cells or muscle cells during moderate or vigorous or exhausting exercise?

Broun (1922) has ventured into this difficult field with commendable courage. There are certain differences in his results as compared with the earlier experiments of Lee, Carrier and Whipple (1922). We are inclined to explain the slight differences in plasma and red cell volumes by various differences in the physical condition and exercise periods of dogs. We noted a slight decrease in plasma volume in contrast to a slight increase in Broun's experiments. We found no change in red cell volume and are not inclined to stress too much the observations of Broun which he interprets as due to blood destruction. Variations in plasma and cell volume are observed in closely confined dogs suddenly subjected to vigorous and perhaps exhausting exercise. Can any person be sure that the muscle hemoglobin does not change under such widely different conditions and absorb more or less carbon monoxide due to the tremendous changes in the muscle fibres?

McMaster, Broun and Rous (1923) have shown a moderate increase in bile pigments during periods of exercise but some of this increase is almost certainly due to increased breakdown of muscle hemoglobin. If some of this bile pigment increase comes from red cell or muscle hemoglobin how much of one or the other may be conserved for reconstruction into new hemoglobin? We know that red cell hemoglobin can be conserved if there

is acute shortage in the body but we do not know whether muscle hemoglobin can be similarly conserved. Much of this information must be at hand before we can talk confidently about red cell destruction as mirrored in the bile pigment excretion.

Feigl 1916 reports an interesting type of hemoglobinuria in troops after long marches, which may be referable in part at least to extreme muscular activity and escape of some muscle hemoglobin from the exhausted muscle cells. Günther (1924) reports hemoglobinuria in man associated with a myositis. These observations indicate that muscle hemoglobin may escape from the muscle bundles under certain abnormal conditions or during extreme exercise.

SUMMARY

Solutions of muscle hemoglobin free from blood hemoglobin introduced intraperitoneally, intramuscularly and intravenously are promptly broken down and at least in part excreted as bile pigment in the urine (dog).

It seems highly probable that the wear and tear or physiological wastage of muscle hemoglobin in the body is a source of bile pigments.

What the relative importance of muscle hemoglobin and blood hemoglobin may be in the production of bile pigment within the body is unknown—a stimulating and disturbing variable.

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STUDIES ON ADRENAL INSUFFICIENCY IN DOGS

I. Control Animals Not Subjected to any Treatment

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In the past ten years we have had many opportunities, in the course of work of various kinds on the adrenals filling practically the whole of that period, to study the pathology and symptomatology of adrenal insufficiency in rats, rabbits, guinea pigs, cats and dogs. References to these observations and certain tentative conclusions and hypotheses based upon them were published from time to time (Stewart, 1921, 1924; Stewart and Rogoff, 1925 a, b; 1926). It may be mentioned once more that whether published for convenience under the name of one or of both of us, all the work has been done in collaboration. It was emphasized as particularly important for the solution of the adrenal problem that animals may for a time remain in good health in the absence of the adrenals, this period being then interrupted by the appearance, usually abrupt, of the characteristic symptoms, followed by death. This has nothing to do with the question of the compensatory function of accessory adrenal tissue, for the phenomenon is seen in animals, like guinea pigs, which seldom possess such tissue, as well as in that large fraction of all rabbits, which do not survive double adrenalectomy. The change for the worse must be due to the breakdown of some mechanism which is keeping the animal alive in the absence of the adrenals. One might think of a store of hormone which becomes exhausted, even some organ which for a limited time can vicariously supply something which takes the place of the adrenal (cortical) hormone, if there is one, or some organ which excretes or neutralizes a poison accumulating in the absence of the adrenals and which eventually ceases to do so sufficiently, perhaps being crippled by the poison itself. These hypotheses, and there are others which may be thought of, will be discussed at greater length later on, particularly in papers which will deal with the blood changes, the influence of pregnancy on the survival period and the favorable influence of injections of Ringer's solution in delaying the development of the intoxication, which, however induced, does undoubtedly develop in adrenal insufficiency.

When, some years ago, having convinced ourselves of the physiological

insignificance of the adrenal medulla, we resolved to concentrate upon the cortex, it was also determined in the first instance to use dogs only. The dog was chosen for several reasons, of which the following are the most important. Blood samples of sufficient amount for analysis can be obtained as often as desired without handicapping the animal. The excreta can also be obtained in sufficient quantity. Symptoms in dogs are more definite than in most laboratory animals and their interpretation is less difficult. It is therefore easier to fix the time of appearance of any abnormal behavior before it has become very pronounced. The psychology of the dog is most suitable. The persistence of acquired habits such as special little tricks, after removal of the adrenals, and their abrupt or gradual disappearance often give valuable information as to the development of the pathological condition. The fact observed by us that no dog survives total loss of the adrenals for an indefinite time, unlike rats and rabbits, renders this animal very suitable for studies on the duration of life after this operation. We have performed the operation on more than 120 dogs and have found no exception to this rule. Nor did careful macroscopic search reveal in any case an accessory adrenal. When, therefore, under treatment or in certain physiological conditions it is found that the animals are living well beyond the limits of the control series, it is not necessary to puzzle over the possibility of the exceptional result being due to an accessory adrenal. Naturally it must be known for certain that the adrenals have been completely removed. The statements in the literature that dogs sometimes survive the double operation indefinitely (many months) we consider to be erroneous. Of course the possibility must be granted that if more dogs are operated on a genuine example of indefinite survival may be encountered, but it becomes less likely as the number of negative results increases.

Duration of survival of the control animals. We soon found that the results given in the literature are of little, if any value, as there is evidence that the dogs usually died not of adrenal insufficiency but of the operation as such (trauma, hemorrhage, anesthetic, cooling, shock, etc.). Practically all the series (including quite recent work) are vitiated by an inadequate technique. It has usually not been sufficiently appreciated that a great amount of practice is necessary to obtain data more nearly representing the truth on the survival period and on the clinical picture when uncomplicated by the effects of the surgical operation as such. Among other things, practice permits the operations to be greatly shortened. The average time now required between the making of the skin incision and the suturing of the wound is 8 to 15 minutes; and there is practically no difference for the two adrenals, in the time and difficulty of the operation. Not a single dog has died of hemorrhage, shock or other surgical accident, although it is to be assumed that among those cases which sur-

TABLE 1

Adrenalectomized male dogs

NUMBER OF DOG	BREED	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION
		First operation	Second operation					Right	Left	Blood	Congestion	
		kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gram*	*gram*			
860				7	2½							
977	Terrier	13.4	14.9	20	5¾		3½	0.65	0.82	++		
980	Fox Terrier	11.5	10.95	12	14	20	13	0.65	0.54	+++	++	++
984	Mongrel	7.6	8.0	7	5½		4	0.66	0.63			
985	Bull	10.6	10.0	10	9½		7	0.69	0.70	++	++	++
986	Fox Terrier	9.0	9.5	12	6	7	3½	0.50	0.50	++	+++	++
988	Bull Terrier	12.0	12.5	11	8		6¼	0.48	0.60	+++	++++	++
991	Fox Terrier	11.2	11.0	12	4	3	0	0.70	0.78	++	+	++
992	Hound	10.6	11.5	25	7	3	3	0.90	0.80	+++	+++	++
995	Spaniel	8.0	8.7	26	7¾		5	0.52	0.62	++	+++	++
998	Airedale	8.4	7.5	18	5¾		4	0.50	0.57	++	+++	++
999	Mongrel	8.0	7.5	17	7	4	5	0.54	0.55	+	++	++
1003	Mongrel	5.5	5.5	23	2½		1	0.52	0.43	+++	+	+++
1004	Mongrel	9.5	10.2	28	4¾		3	0.63	0.66	+++	+	+++
1007	Mongrel*	8.3	7.8	12	3½		2½	0.57	0.60	0	0	+++
1008	Poodle	8.5	8.2	12	9½		7	0.49	0.55	+++	+++	+++
1012	Terrier	5.6	5.8	34	6¾		5½	0.45	0.44	++++	++++	++++
1014	Terrier	8.5	8.0	35	5	2	2	0.55	0.61	+	+	++
1015	Airedale	4.5	4.6	14	4¾		3	0.59	0.37	0	+	+
1016	Airedale	3.7	4.3	14	3½		2	0.25	0.32	0	0	+
1019	Dachs	8.8	9.0	14	9		8	0.52	0.65	++++	++++	++++
1023	Mongrel	7.0	7.8	68	8½		4	0.52	0.65	++	+	+++
1033	Mongrel	6.1	7.8	56	10		7	0.50	0.44	+++	+++	+++
1037	Mongrel	8.3	8.1	9	9½		7	0.59	0.46	++	++	++
1039	Poodle	7.5	6.6	28	7			0.50	0.40	0	0	++
1136	Collie	9.1	10 3	51	4¼		2½	0.50	0.52	+++	+++	++
1151	Collie	8.1	6.6	20	7½		5½	0.53	0.65	0	0	0
1158	Fox Terrier	8.6	8.2	7	14	11	12	0.47	0.50	+++	+++	++++
1169	Poodle†	8.3	8.5	10	8	16	2½	0.56	0.62	+++	+++	+
1175	Poodle	5.5	6.3	134	6¼		4	0.62	0.62	++++	++++	++++
1189	Mongrel*	8.0	8.2	50	8	5	6	0.50		++++	++++	++++
1195	Fox Terrier*	7.5	8.9	122	3¾		2	0.70	0.72	++	++	+++
1200	Terrier	9.7	9.4	17	7	3½	4	0.48	0.66	++++	++++	+++
1204	Hound	12.5	12.7	17	6	11	4	0.80	0.84	++	++	++++

* Mangy.

† Wound superficially infected.

In dogs 1015, 1032, 1033, 1037, 1039 and 1136 the right adrenal was removed last, in all the others first.

vived for relatively short periods there were some which did not die solely of adrenal insufficiency, but in which the operation as such was a contributory cause. Be that as it may, all the animals survived more than 24 hours. We have not seen any dying in a few hours, as is so commonly stated in the literature. The average survival period for the 34 male dogs (table 1) is 6.9 days, and for the 39 non-pregnant females (table 2) 6.4 days. The average for the 73 control dogs taken together is just $6\frac{2}{3}$ days. On account of the rather wide range in the duration of survival in different dogs these averages have only a limited value. Yet a glance over the tables shows that most of the animals do survive from 4 or 5 to 8 or 9 days, a smaller fraction living longer (even occasionally into the 15th day) and another smaller fraction dying in less than 4 days. As stated, we think that when dogs die in 2 or 3 days or less something else than adrenal insufficiency has contributed to shorten life, especially the direct consequences of the surgical procedure. But of course, no animal has been excluded from the tables for this reason. A few were excluded because there were obvious intercurrent causes of death, but all of these are reported. Since the manuscript was sent in one more control animal (a non-pregnant female) has been added, making 74 in all. She died on the 12th day after removal of the second adrenal.

We cannot estimate how far good hygienic conditions have favored our animals. We are inclined to attribute considerable importance to the prevention of cooling at the operation, and the keeping of the dogs in warm quarters for some time thereafter. A bath before operation is part of the routine. The dogs receive no food for 24 hours before the operation and are not fed till the day after. Recovery from the operation is a matter of an hour to as little as 15 minutes. The dogs are then on their feet, often jumping up in the cage and ready to eat, if permitted. Water is withheld for the first few hours. Since they enjoy a period of good, and in the great majority of cases of completely normal health before the symptoms associated with adrenal insufficiency appear, it is easy to separate the consequences of the surgical procedure from those of the loss of the glands. The study of the clinical condition can be followed at leisure as the symptoms develop at the end of the period of normal health. The same is true of the study of the blood and excreta.

Nothing can be less promising than to make clinical and chemical studies on animals surviving only a few hours, as has been done in a series of recent papers by Bornstein (and collaborators) (1923, 1925). These were apparently the best of their animals, the others dying practically at once. They determined the total ventilation, respiratory quotient, alveolar CO_2 tension, alkali reserve of blood, acidity of urine, ammonia-quotient of urine, and Hb content of blood. They consider overventilation as a specific consequence of adrenal insufficiency. It seems to

us useless to make such observations on moribund animals with the object of studying the effects of removal of the adrenals. The "over-ventilation" is simply the terminal dyspnea, and in our opinion is not a specific symptom at all. In any case it is impossible to make a study of the clinical conditions induced by complete loss of function of the adrenals on such material, i.e., by investigating the terminal stages in animals dying entirely or mainly from surgical causes. As already stated, animals surviving only a few hours were not encountered by us in our series of more than 120 dogs. Not one died in as short a time as 24 hours. Removal of both adrenals at one operation, as in Bornstein's experiments, has nothing in its favor and much against it.

In still more recent observations by Blodinger, Klebanoff and Laurens (1926), the great majority of the dogs died in 8 to 24 hours, although the operation was performed in two stages. Two of the animals lived about 4 days. These results are not as good as those of some of the older series.

As regards our own technique, all that need be added at present to what has been said is that the operation, always in two stages, was performed extra-peritoneally through a lumbar incision under ether. The interval between removal of the two adrenals varied from about a week to 19 weeks. After a week the results were as good as after longer periods.

For convenience some of the results of the chemical examination of the blood (on about 15 of the control dogs), comprising estimations of the total non-protein N, urea N, creatine and creatinine, uric acid, amino-acid N, undetermined fraction of non-protein N, chlorine and dextrose, will be given in paper II. The estimations were made on blood samples drawn before the second adrenal operation and at different times thereafter. The object was to obtain samples during the period of good health, and while the symptoms were developing, and in the terminal stages. Sometimes blood was also obtained before the first operation. A table has been published in another place (Stewart, 1926) giving results on 20 dogs, in which estimations were made from time to time of blood sugar, serum proteins (refractometer and specific gravity), conductivity (of blood and serum), relative volume of erythrocytes and serum, hemoglobin percentage with counts of erythrocytes and leucocytes, etc. These results will be discussed later on.

It should be explained that the reason for including only non-pregnant females in the control series is that, as we have observed, pregnancy tends to increase the survival period (to as much as 6 or 7 times the average period). This matter will be taken up in paper III.

The headings of the various columns in tables 1 and 2 are self-explanatory. As regards breed, most of the dogs were of course mongrels. The predominating type is indicated as far as possible. A few Airedales were pure bred. The body weights at the time of the two adrenal operations

TABLE 2

Adrenalectomized female dogs (non-pregnant)

NUMBER OF DOG	BREED	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION
		First operation	Second operation					Right	Left	Blood	Congestion	
		kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gram*	*gram*			
859				7	4½		3					
974	Spaniel	9.5	9.0	7	2	7	1¼	0.62	0.70			
987	Terrier	7.5	8.5	9	5½		4	0.52	0.62	++	++	
993	Mongrel	10.6	10.3	25	3½		2	0.67	0.70	+++	+	++
1001	Fox Terrier	8.3	7.4	17	8¾		7	0.42	0.55	+++	+++	++
1009	Mongrel*	8.9	8.5	8	5	4	3½	0.64	0.72	±	+−	+++
1010	Mongrel	10.5	11.5	40	7		4½	0.50	0.55	++	+++	++++
1013	Terrier*	9.3	9.7	35	7	5	5½	0.48	0.60	+++	+++	+++
1018		5.6	6.1	35	7	8	5	0.48	0.54	0	+	++
1020	Bull Terrier	6.8	6.7	17	10	9	8½	0.70	0.72	+++	+++	+++
1022	Fox Terrier	5.6	8.6	78	10		8	0.36	0.53	+++	+++	+++
1025	Fox Terrier	7.3	9.1	36	9½		8	0.35	0.50	+++	+++	+++
1027	Shepherd	10.8	10.9	10	4¾		3½	0.89	0.77	+	+	++
1028	Airedale	12.4	12.4	10	7½		6	0.90	0.68	+++	+++	+++
1031	Fox Terrier	8.8	8.6	11	3	10	0	0.52	0.37	++	++	+++
1038	Bull	9.2	8.6	7	9	4	7½	0.68	0.62	+++	+++	+++
1126	Airedale	12.9	11.9	64	4¾		2	0.95	0.85	++	++	++++
1138		10.6	10.9	55	6	10½	5	1.02	0.80	+++	+++	++++
1145	Mongrel	13.0	12.4	38	4	7	3	0.85	0.85	++	++	++
1146	Collie	8.9	9.7	186	7	3¼	6	0.55	0.75	+	++	++++
1148	Bull	11.5	11.4	23	11½		9½	0.85	0.86	++	++	+++
1149	Collie	10.3	12.1	190	8	8½	6	0.65	0.70	+++	+++	++++
1150	Mongrel	10.8	10.5	23	5	10	4	0.70	0.70	+	+++	++++
1154	Mongrel	7.2	7.5	15	9	½	7½	0.50	0.43	++	+++	++++
1156	Collie	8.9	8.3	11	3½			0.80	0.65	++	++	++++
1160	Bull	10.1	9.4	25	4	1	2	0.57	0.55	++	++	+++
1161	Collie	8.1	7.9	23	7	7	6		0.63	0	++	++++
1163	Mongrel	8.7	7.9	22	10	8	7	0.65	0.65	++	++	++
1171	Airedale	9.9	9.4	6	4	8	3			0	+−	+++
1180	Collie	13.0	12.0	78	9	2½	7	0.82		+	0	+++
1181	Mongrel*	6.7	6.3	27	7	2	5½	0.30	0.40	0	0	0
1182	Mongrel	8.7	10.2	109	10	18	9	0.60	0.75	+++	++++	+++
1186	Bull Terrier	8.5	11.0	127	10½		8½	0.55	0.80	0	+	++++
1188	Airedale	9.5	10.0	78	6	20½	5½	0.75	0.80	+++	+++	++++
1196	Collie	11.3	12.7	69	3	17	2	0.52	0.75	+++	+++	++++
1197	Poodle	8.9	7.9	48	3	17	2	0.48	0.57	0	+	+++
1201	Spaniel	13.5	13.4	20	2	18½	0	0.55	0.55	++++	++++	++++
1202	Shepherd	7.0	8.6	15	1½		0	0.68	0.70	+	0	++
1203	Mongrel*	7.5	7.4	15	2½		0	0.44	0.43	+−	0	+++

* Mangy.

In dogs 1027 to 1138 the left adrenal was removed first, in all others the right.

are given to illustrate the fact that the animals continued in good health after removal of the first adrenal. Weights were also taken at other times and after removal of the second adrenal but cannot be given in the tables. Not infrequently some weight was put on, especially of course in the case of young animals; e.g., in dog 1022 (table 2) the weight increased from 5.6 kgm. to 8.6 kgm. in the 78 days between removal of the first and second adrenals. Rarely is there any significant diminution of weight. In dog 1151 (table 1), however, the weight declined from 8.1 to 6.6 kgm. in a 20-day interval. During the first part of this period the dog (with one adrenal out) was used as a control on the effect of daily intravenous injections of Ringer-dextrose solution. Whether this was connected with the injections or not, there was some diarrhea, loss of appetite, and vomiting; and after 7 days the injections were discontinued. He was then some time in quarantine with cough and some nasal discharge. As already mentioned, an interval of a week was found to give as good results for the second operation as a longer one. At the end of that time healing of the small wound (by first intention) was complete.

Anorexia. Refusal of food at the regular feeding time, particularly of the kind of food of which the animal has hitherto been fond, is the most definite and in the great majority of cases the earliest symptom, and indicates that the period of good health is over, or is about to terminate. There is seldom any ambiguity about this change. If the column giving the time of survival be compared with that giving the time up to the refusal of food, it will be seen that the animals which live a relatively long time also enjoy a long period of good health. Thus, the two dogs 980 and 1158 (table 1) survived 14 days 20 hours and 14 days 11 hours respectively (much the longest periods observed). The appetite remained good and so did the general condition, for 13 days and 12 days respectively. The same thing is seen among the non-pregnant females (table 2). Dogs 1020, 1022, 1148 and 1182 lived 10 days 9 hours, 10 days, 11½ days, and 10 days 15 hours respectively after removal of the second adrenal; while the appetite remained normal for 8½ days, 8 days, 9½ days and 9 days respectively. In rare cases the animal was never seen to eat after the second operation. There is one case in table 1, indicated by "O" in the appropriate column, viz., dog 991, 4 cases in table 2 (dogs 1031, 1201 to 1203). With the shorter periods of survival (2½ to 4 or 5 days) the period of normal health and good appetite being relatively less than with the longer periods of survival, the time for which the dog still lives after the anorexia has declared itself is not very different in the great majority of the animals whether the total survival period be long or short. For example, in table 1, most of the animals died in less than 3 days after refusing food. Not more than one-sixth survived more than 3 days after anorexia developed. If it be remembered that the time at which food is

definitely refused is determined by offering food when the usual interval from the last meal has elapsed (generally 24 hours), the general uniformity in the residual survival periods obtained by deducting the numbers in the seventh from those in the sixth column is significant. Thus, in table 1 we get $2\frac{1}{4}$, $1\frac{5}{6}$, $1\frac{1}{2}$, $2\frac{1}{2}$, $2\frac{5}{6}$, $1\frac{1}{2}$, $4\frac{1}{8}$, $4\frac{1}{8}$, $2\frac{3}{4}$, $1\frac{3}{4}$, $2\frac{1}{6}$, $1\frac{1}{2}$, $1\frac{3}{4}$, 1, $2\frac{1}{2}$, $1\frac{1}{6}$, 3, $1\frac{3}{4}$, $1\frac{1}{2}$, 1, $4\frac{1}{2}$, $5\frac{1}{2}$, 3, $2\frac{1}{2}$, $1\frac{3}{4}$, 2, $2\frac{1}{2}$, $6\frac{1}{3}$, $2\frac{1}{4}$, 2, $1\frac{3}{4}$, 3, $2\frac{1}{2}$. In table 2 the corresponding numbers are 1, $1\frac{1}{2}$, $1\frac{1}{2}$, $1\frac{3}{4}$, $1\frac{2}{3}$, $2\frac{1}{2}$, $1\frac{2}{3}$, $2\frac{1}{3}$, 2, 2, $1\frac{1}{2}$, $1\frac{1}{6}$, $1\frac{1}{2}$, $3\frac{1}{2}$, $1\frac{2}{3}$, $2\frac{2}{3}$, $1\frac{1}{2}$, $1\frac{1}{3}$, $1\frac{1}{8}$, 2, $1\frac{1}{2}$, $1\frac{1}{2}$, $3\frac{1}{2}$, 2, $1\frac{1}{3}$, $1\frac{1}{3}$, $3\frac{1}{3}$, 2, $1\frac{1}{2}$, $1\frac{3}{4}$, $1\frac{1}{3}$, $1\frac{3}{4}$, $2\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{2}$. It must be repeated that the fractions have no absolute value, as a dog's behavior when food is offered him depends upon his individual tastes and upon the time, nature and quantity of the meal. It is nevertheless remarkable how sharply the time of onset of the anorexia can in the great majority of the animals be fixed by an observer with some experience. We have found it a good plan, in addition, of course, to observing the animal at the usual time of feeding, to offer him a small portion of food which he is known to relish (e.g., half of a dog biscuit) at the first visit in the morning and the last at night. The test does not then interfere with the routine feeding of the animal. The loss of appetite comes on so abruptly and is so complete and permanent that the diagnosis has hardly ever to be revised, unless occasionally in pregnant animals or in animals kept alive by treatment, in which during the relatively long period of survival appetite may sometimes be temporarily lost.

In two columns of tables 1 and 2 are given the weights of the two adrenal glands. In our routine these are always weighed as soon as the operation is completed. One reason for weighing them is to obtain evidence on the development of so-called compensatory hyperplasia (or hypertrophy) in the interval between the two operations. In all the animals, with the exceptions specified at the foot of the tables, the right gland was removed first. The tendency to compensatory adrenal growth in the dog does not seem to be at all as marked as in the rabbit or guinea pig, although in such a case as dog 1146 (table 2) some hypertrophy did occur in the long period of 186 days. In dog 1175 (table 1) there seemed to be no hypertrophy in 134 days. It must be remembered that sometimes an apparent increase in the weight of the second gland may be associated simply with the growth of a young animal, as possibly in 1022 and 1025 (table 2).

The development of the other symptoms besides the anorexia cannot be shown in the tables, but can only be illustrated by the citation of some of the protocols. In the last three columns of tables 1 and 2 an attempt is made to represent the intensity of the congestion (often accompanied by hemorrhage) of the mucosa of the whole or part of the gastro-intestinal tract and of the pancreas, and the quantity of blood or blood pigment present in the contents of one or more portions of the alimentary canal.

Whatever their significance, these changes, as may be seen from the tables, are very common and often very marked. Rarely indeed are they completely absent. In one case (dog 1181, table 2) the lack of congestion was possibly due to intravenous injection shortly before death, of Ringer-dextrose solution which improved the circulation. In dog 1151 (table 1) there had been some respiratory involvement and nasal discharge, with loss of weight and decided emaciation. It was only because the autopsy showed no other apparent cause of death that it was decided to include the dog among the controls dying of adrenal insufficiency. Dogs 1015 and 1016 were Airedale pups of the same litter. They both developed severe "mad" symptoms after removal of the second adrenal and survived for much less than the average time. Whether this had anything to do with the almost negative pathological picture in the gastro-intestinal tract is unknown.

Autopsy findings. It will be convenient at this point to describe in greater detail the pathological changes seen *post mortem*. The congestion of the pancreas, usually intense, rarely absent, has seemed to us to be fully as characteristic as the changes in the stomach and intestines. That it is not entirely a passive congestion associated with gradual failure of the circulation, engorgement of the right heart, and of the liver and kidneys, seems to be indicated by the fact that although the liver and kidneys are often much congested, the greatest congestion in the pancreas may coincide with a moderate degree of engorgement in these organs and *vice versa*. While the spleen is sometimes much congested, frequently this is not the case, and the greatest congestion in the pancreas may coincide with little or none in the spleen. There is also no close parallelism between the condition of the gastro-intestinal tract and that of the pancreas in this regard. Microscopic examination shows dilatation of the small vessels in the islets as well as marked congestion of the veins in the trabeculae. In some of the cases practically the whole gastro-intestinal mucosa from the cardiac end of the stomach is highly congested, with numerous hemorrhagic patches and blood in the lumen. Sometimes the hemorrhages on or in the mucosa are of good size, perhaps confined to one part of the tube, as the stomach, or a portion of the jejunum-ileum or the colon and rectum; often they are minute and widely scattered. In the stomach the pyloric end is often free from congestion and hemorrhage while the rest of the viscus may be involved. In the colon and rectum the changes are apt to be most marked on the longitudinal ridges and the ileo-colic valve. The caecum and its process are often free from congestion and hemorrhage when the colon and ileum are congested and have blood in their lumen. But sometimes the caecum and process are congested and contain blood, and occasionally blood is present without any congestion. The fact that the caecum and especially the process are often

free from congestion and their contents from blood (e.g., in dogs 1196, 1204) while the rest of the bowel higher up and lower down may be much congested, and hemorrhagic and contain blood, seems to us to suggest that the condition in the gastro-intestinal tract is not the result of passive congestion but possibly the result of an intoxication of the mucosa. This, whether due to the entrance of toxic material from the lumen of the gut, or as seems fully as likely, to an attempt to eliminate such material through the mucosa, would be expected to affect especially the portions of the intestine physiologically the most active. Occasionally the hemorrhagic condition may involve the cecum and vermiform as well as the rest of the intestine. The esophagus is never involved. If bloody contents are present in it they have come from the stomach.

Microscopically the congestion (and hemorrhages) are seen to be confined to the mucosa of the stomach or intestines. The depth of mucosa which is involved varies much in different cases; sometimes only a narrow zone next to the lumen being affected and in the small intestine only the tips of the villi. Sometimes for the whole depth of the villi the vessels are greatly distended, and in other cases the congestion is evident in the vessels between the crypts right down to the muscularis mucosae. The amount of hemorrhage into the lumen is not in proportion to the depth of mucosa affected. In some of the cases which showed most blood in the lumen the changes in the mucosa were chiefly confined to the surface.

The stomach practically always contained bile, sometimes a large amount. If blood was present it was in addition to bile. This is true also of the small intestine. As the animals did not eat for some time before death feces were usually scanty or absent. The blood was often so little digested that erythrocytes could be readily recognized in the small clots. This was perhaps especially true of the rectum when it was hemorrhagic, which was often the case. The spectrum of oxyhemoglobin was usually obtained on rubbing up with water some of the contents of any part of the intestine, and often in those of the stomach; also frequently when contents were allowed to settle in a test tube or were filtered. Usually still more blood pigment was present in the form of hematin, as shown by spectroscopic tests, e.g., conversion to hemochromogen. The feces often contained blood or blood pigment in the last days of life, the vomit much less frequently. Sometimes the demonstration was obtained that some blood was being passed into the intestine at a particular time, by giving castor oil or a colonic injection. The temporary presence of a certain amount of blood, however, in the feces of dogs kept in the laboratory with one or both adrenals intact, is not so very uncommon (perhaps sometimes due to worms), and significance is attached to the symptom only because it is more frequent in the animals with adrenal insufficiency, and because it is often clearly associated with the presence of considerable

quantities of blood in the gastro-intestinal lumen. However, the amount of blood passed is by no means always an index of the severity of the congestion which will be found *post mortem*, and which may be intense without any blood having appeared in the feces.

We have recently had the opportunity of examining the gastro-intestinal tract and adrenals in a case of Addison's disease (a woman 35 to 36 years old) at St. Alexis hospital. She entered the hospital in the forenoon with a blood pressure of 64 (systolic), 56 (diastolic). She died at 4 p.m. and an autopsy was done at 7 p.m. Only a small abdominal incision was permitted. There was no skin pigmentation, except a slightly darkened ridge at the margin of the mucosa of the lips. The adrenals were both reduced to completely fibrosed masses. In the lungs were numerous clumps of tubercles. The stomach was full of watery, bile-colored liquid; the mucosa was moderately congested, with numeous petechial hemorrhages throughout the fundus. There were no ulcers. The duodenum showed decided congestion (++), bile-stained contents, no ulcers. The jejunum-ileum mucosa was increasingly congested from above (+) downward (++) with hemorrhagic areas throughout. The contents were semi-liquid fecal matter (bile-colored). The large bowel had bloody contents, and the mucosa was much congested (++++) and hemorrhagic down to and including the rectum. The pancreas was congested (++).

In the adrenalectomized dogs ulcers were rather frequently seen in the stomach or duodenum or both—in at least one-sixth of the cases (e.g., in dogs 1014, 1026, 1032, 1038, 1195, 1196 and others). The ulcers noted were in autopsies made immediately after death, as was the case in the great majority of our autopsies. In the duodenum ulcers are apt to be situated towards the pyloric end about the orifice of the main pancreatic duct. Hemorrhagic congestion is also apt to be seen here. In connection with the autopsies it should be stated that they were always complete for the thorax and abdomen, including the site of the wound of the second operation and the sites of the excised adrenals. Careful search was made for possible accessory adrenals. Specimens were kept for gross and microscopic examination. In the protocols cited only *post mortem* findings which seem significant will be given. When organs are not mentioned they can be assumed to have shown nothing abnormal. Females classified as non-pregnant were always known to be so from the autopsy findings.

To obtain a standard by which to judge the significance of the pathological findings described autopsies were made on 17 supposedly normal dogs. Two of these had been used for experiments in the Laboratory of Physiology and had received chloretone, and one chloralose intraperitoneally. They were allowed to die of anoxemia. None of these showed any congestion of the pancreas or any blood in the gastro-intestinal contents. The first two had considerable congestion in the duodenum, and the jejunum also showed some congestion. Ten dogs received heparin

and were kept under ether for 2 or 3 hours in experiments in this laboratory, and were then killed by asphyxia. None of them had any congestion of the pancreas, or any blood in the gastro-intestinal lumen, or any significant congestion of the mucosa. In one of the dogs there was an extravasation of blood into the mucosa about four-fifths down the small intestine. The cause of this is unknown. It may have been traumatic as the bulk of these so-called normal dogs used for acute experiments, unlike those for adrenalectomy, were obtained from a general kennel and some had recently made a journey. Further, in this particular dog the abdomen had been opened and the intestines and stomach exposed. Four dogs, supposed to be normal, were sacrificed solely for autopsy. Of these two showed no congestion of pancreas or gastro-intestinal mucosa, and no blood in the contents. In one the pancreas was not congested;

TABLE 3

Non-adrenalectomized dogs

NUMBER OF DOG	ALIMENTARY CANAL		PANCREAS CONGESTION	NUMBER OF DOG	ALIMENTARY CANAL		PANCREAS CONGESTION
	Blood	Congestion			Blood	Congestion	
I	0	0	0	XIII	0	+	0
II	0	0	0	XIV	0	0	0
III	0	0	0	XV	0	+	0
IV	0	0	0	XVI	0	++	0
V	0	+	0	XVII	0	++	0
VI	+−	0	+	XVIII	+−	+	+++
VII	0	0	0	XIX	0	0	0
VIII	0	0	0	XX	0	0	0
IX	0	+	0	XXI	0	0	0
X	0	0	0	XXII			0
XI	0	++	+	XXIII	0	0	0
XII	0	0	0				

blood was absent from the canal, but there was slight uniform congestion (without hemorrhages) in the lower half metre of the small intestine. In the fourth dog, obtained from the general kennel, the pancreas was slightly congested, and the upper half of the small intestine, including the duodenum, much congested. There was no blood in the lumen. A clinical history of the animal was not obtainable. The *post mortem* observations on the pancreas and gastro-intestinal tube in these 17 non-adrenalectomized animals (dogs I to XVII) are assembled in table 3, in which are also included 6 dogs (dogs XVIII to XXIII) suffering from various diseases. Some of these were allowed to die; the others were killed. Four of them were pups (XVIII, XIX, XXII, XXIII).

The only one of these 6 dogs which showed any decided pathological change was XVIII, the pancreas being much congested. The animal,

which had been previously in good health had severe convulsions the day before death. Worms being suspected, castor oil was given and a bowel movement followed. At autopsy only 3 small round worms were found. More than half of the right lung was hepatized. The spleen was congested. The kidneys showed nothing abnormal. The stomach contained a ball of "excelsior," and there were a few small congested spots near the pyloric end; a few similar spots in the duodenum and 3 small hemorrhagic patches in the upper end of the ileum. The contents of the small intestine gave a weak but definite hemochromogen spectrum (with KOH and $(NH_4)_2S$. Dog XIX, a pup, had a "mad" fit and was killed. The autopsy showed nothing abnormal. Dog XX, a stock female, died of pneumonia (double), as shown by autopsy. There was no congestion

TABLE 4

Adrenalectomized dogs not included in tables 1 and 2

NUMBER OF DOG	SEX	BREED	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		ALIMENTARY ANAL		PANCREAS CONGESTION
			First operation	Second operation				Right	Left	Blood	Congestion	
			kgm.	*kgm.*	*days*	*days*	*hours*	*gram*	*gram*			
1021	F	Fox Terrier	12.1	11.0	10	1½		0.67	0.80	+	+	++
1024	M	Mongrel	9.2	9.6	36	3	1	0.73	0.76	++	+	++
1030	M	Airedale	9.7	9.1	11	1⅞		0.85	0.66	++	0	+
1167	M		4.0	5.0	12	2	1	0.37	0.35	+	+	++++
1168	F	Collie	10.4	11.0	10	2¾		0.55	0.67	+	0	+++
1194	F	Collie	9.5	8.5	5	1	3	0.74	0.80	+−	0	+

In dog 1030 the left adrenal was removed first, in the others the right.

of the pancreas, spleen, omentum, or alimentary canal, and no blood in the contents, although the day before death there was blood in the feces. Dog XXI, a stock female, had been coughing for 3 weeks. The dog was killed by a blow. At autopsy both lungs showed disseminated areas of edema and congestion with some small consolidated patches. The abdominal viscera showed nothing special. In particular there was no congestion of the pancreas or alimentary canal. XXII was a pup of dog 1002 (see paper IV) born 16 days after removal of the second adrenal. It was fed for a time after the mother's death and throve, but was probably neglected later on. It died, the autopsy showing nothing special; the stomach contained curdled milk. The pancreas was not congested. The pup weighed 255 grams and the two adrenals together 0.090 gram. XXIII was a very young pup, dying of an unknown cause. It was killed.

The upper part of the small intestine was full of round worms. There was no congestion of pancreas or gastro-intestinal tract and no blood or blood pigment in the lumen. It will be seen that the findings displayed in table 3 form a complete contrast to those in tables 1 and 2.

A small number of dogs dying after removal of the second adrenal have not been included in tables 1 and 2 because the symptoms and *post mortem* appearances indicated that they died of something else than adrenal insufficiency. Sometimes they were operated on in order to get rid of them, as they were not thriving. It would be improper to include them. But it is all the more necessary to report them, so that any reader who chooses to deal otherwise with them than we have done may do so. Up to the present there are in all 6 of these cases, which are assembled in table 4. In all except 1030 the right gland was excised first.

Dog 1021, from the condition of the uterus, must have had a recent parturition. There was an excellent post-operative recovery after removal of the right adrenal. Nine days thereafter the blood sugar was 0.10 per cent. On the 10th day the left adrenal was excised, but there was unusual hemorrhage from an anomalous vein and trauma, and the post-operative recovery was slow. No food was taken. The dog remained apathetic, though not weak. An injection of Ringer-dextrose was given with temporary benefit, but the animal died about 1½ days after the second operation. The wound was clean. The chest and abdomen contained some blood-stained fluid; pancreas and omentum congested; kidneys moderately congested; small hemorrhages in gastric mucosa. The stomach and duodenum contained a small amount of blood-stained liquid. The spectroscope showed no oxyhemoglobin spectrum in contents of stomach, duodenum or jejunum-ileum, but a good hemochromogen spectrum with KOH and $(NH_4)_2S$.

Dog 1024. In the removal of the second adrenal (left) there was much trauma and some hemorrhage. The respiration under the anesthetic was unsatisfactory, there being much mucus in the bronchi. The post-operative recovery was slow. Although the animal was able to walk all right when roused, it lay about, as if ill, and took no food. It died just over 3 days from the second operation. The wound on the left side was found infected in the muscular layer, with local peritonitis, although there was no pus in the peritoneal cavity, which contained a small amount of bloody fluid. The spleen was very large and congested; liver congested. The other details are given sufficiently in table 4. The day before the second operation the red cell count was 6,920,000; leucocyte count 12,000; Hb. 98 per cent (always by the scale of Haldane's hemoglobinometer); blood sugar 0.095 per cent; conductivity of serum, K (25°) $\times 10^4 = 131.0$; serum in 100 cc. blood 56.9 cc. A few hours before death the erythrocyte count was 8,420,000; leucocytes 29,000; Hb. 116 to 118; blood sugar 0.08 per cent; serum conductivity K (25°) $\times 10^4 = 118.0$; serum in 100 cc. blood 42.3 cc. There has accordingly been a considerable concentration of the blood.

Dog 1030 had cataract in both eyes. The recovery after the two operations was rapid; after the second (right adrenal) he was walking about his cage within 10 minutes, and in 10 minutes more without staggering. But he ate nothing; there was discharge from the nose and symptoms of "snuffles." He lived only 1¾ days, during which time he ate nothing and was obviously in poor condition. The esophagus at autopsy contained bile-colored liquid and several round worms. Many

similar worms in stomach, which contained brownish, foul-smelling liquid, neutral to litmus and giving a strong hemochromogen spectrum with KOH and $(NH_4)_2S$. A good deal of blood in the contents of the lower jejunum and ileum; little, if any, actual congestion of gastro-intestinal mucosa when contents washed off. Pancreas only slightly congested. The bladder was full of urine, which gave a good reaction for bile pigments (Gmelin). This is noted as it was quite unusual in dogs dying after adrenalectomy to obtain a positive Gmelin's test in the urine.

Dog 1167, a pup, had yelling fits for a long time (before the first adrenalectomy and since also). A few hours before the second operation he yelled fiercely and raced about in his cage. The operations were both easy, the actual removal of the glands requiring only 3 or 4 minutes for the right, and 5 minutes for the left adrenal (about 12 minutes' anesthesia). Six hours after the second operation, he had a violent "mad" fit (circus movements always to the right and loud yells). No food was taken. Next day the animal walked poorly; wobbly in the hind legs. On the forenoon of the second day after the last operation, the dog had a violent convulsion. He was comatose for several hours, and died 49 hours after removal of the second adrenal. The pancreas was intensely congested. A large mass of excelsior was found in the stomach with some bile. Later on the dogs slept on smooth wooden platforms and had no bedding. The middle portion of the small intestine was decidedly congested in parts, and contained blood and bile. The liver was much congested, the spleen not. There were many worms in the small intestine.

Dog 1168 made a good recovery from the second operation and ate well in the afternoon (being fed by mistake). Next day it was vomiting chocolate-colored, creamy liquid, strongly acid to litmus and containing much blood. Food was refused. The vomiting of chocolate-colored fluid continued, and the dog died about 66 hours after the second operation. She was well advanced in pregnancy, although not nearly at full term and 6 pups were found in the uterus. No milk could be squeezed from the nipples. The stomach contained about 20 cc. of liquid similar to the vomit, with one or two round worms; no special congestion. The presence of blood was confirmed by the spectroscope. The small intestine contained many worms, but there was no congestion and no blood in the contents nor in the large intestine. The pancreas was much congested. Liver and kidneys somewhat congested, spleen not.

Dog 1194 had been in quarantine with a respiratory infection, and was operated on to get rid of her. After the first operation bloody discharge from the nose continued, and the respiratory infection continued after the second operation. In removal of the first (right) adrenal there was considerable trauma; the cava was punctured, but the puncture was closed by a ligature, and a normal recovery was made. The day after the second operation the noise of a struggle in the room was heard, and it was found that either in a convulsion or in some other manner the dog had rolled from her bed so that her nose dipped into the water in the drinking pan. Apparently she was drowned in this way, 27 hours after removal of the second adrenal. The autopsy (made at once) showed double pneumonia; water in the trachea and bronchi. The stomach contained bile and some blood. There was no congestion anywhere in the alimentary canal, but many worms. The pancreas was pale at the splenic end, with markedly congested blotches in the duodenal portion. This appearance was not seen in any of the other dogs examined by us. The left kidney was congested, the right not.

Symptomatology. It has been already mentioned in describing tables 1 and 2 that the most universal and characteristic symptom in dogs dying

after removal of both glands is the abrupt onset of anorexia. Often this is so definite and so irrevocable, that the animal after the first refusal of food, particularly of food which it has hitherto relished, never eats again. But in a good many cases after the refusal of the ordinary food, a daintier meal will once or perhaps twice tempt the appetite before the animal reaches the stage where it refuses everything.

The next most common symptoms are also centered about the digestive system: vomiting, sometimes persistent, sometimes occasional, the vomit practically always, sooner or later, and often rather early, containing bile. Blood may sometimes be present, but rarely in the earlier stages. Liquid or semi-liquid stools may be passed, and more frequently contain blood.

Somnolence preceded by yawning and stretching is common after the loss of appetite has declared itself, and sometimes in advance of that symptom.

Asthenia has been overemphasized by some writers, who have only witnessed the terminal stages of adrenal insufficiency. Certainly toward the end the dogs are apt to become weak and if capable of walking do so with the legs, especially the hind ones, widely separated; and the movements are ill coördinated. But dying animals are apt to be weak. Often even after the anorexia has persisted for some time the animals are still strong on their legs, capable even of running well and with well coördinated movements. They may need some inducement before making the effort, since their tendency is to lie around or to sleep more than usual and they are not willing to walk although they can do it. Lest these remarks should be misinterpreted we must state that we do not doubt that asthenia is often well-marked in these animals, and of course always as death approaches. But it is not nearly as characteristic or common a symptom as others in those earlier stages where there is still opportunity to observe the clear development of the symptomatology, necessarily obscured in the terminal stages. Certain attitudes are apt to be assumed by the dog in the last day or two. One of the most characteristic is standing in one position with the head and tail down, the brow knit as if careworn, and the general expression what is best described as "down and out." Somewhat earlier in a certain number of cases the dog will try eating a morsel, but the jaw movements are too weak.

Less constant symptoms, sometimes very marked, but often absent, are those connected with the nervous system (convulsions, hallucinations, "mad fits with yelling," etc.). A period of coma, often, or perhaps always, precedes death, but there is nothing characteristic about it. Probably more interesting is the heightened reflex excitability, not necessarily associated with convulsions, which is often seen in the terminal stages. Circulatory changes are doubtless often to be detected sooner or later (slower flow, as seen in drawing blood from a vein, lowered blood pressure,

irregularity of the heart, passing off and recurring perhaps many times in the hours before death, etc.).

Lowered blood pressure has often been spoken of as a characteristic symptom after removal of the adrenals and attributed to loss of the epinephrin output. We think there can be few observers who now believe that epinephrin from the adrenals is an appreciable factor in maintaining the normal blood pressure. For us such belief is impossible, after studying hundreds of animals in which the epinephrin output was suppressed or greatly diminished by various operations not involving removal of the whole of the cortex in cats and dogs, or after total adrenalectomy in many rats and rabbits. These animals live indefinitely in good health and are normal in vigor, activity, capacity to resist fatigue, combativeness, and capacity of procreation. No difference can be seen in the psychology of the animals from what it was before the operations, or from that of unoperated animals. The physiological reactions which ordinarily accompany emotional excitement of various kinds are seen without change in nature or intensity. In short the observer who studies the animals is quite unable to say from their behavior whether they have been operated upon or not. The same is true of the dogs subjected to total adrenalectomy in the present research. During the period of good health when the animal is eating well, running around, jumping and playing with other dogs, interested in sexual matters, combative if he had been so before the second operation, it is certain that he is not suffering from abnormally low blood pressure. Rogoff and Dominguez (1924) made systematic measurements of the blood pressure by means of the carotid loop method in a number of rabbits, beginning before excision of the first adrenal and continuing regularly thereafter. The observations were made even during the two operations. They obtained no evidence that the pressure was at all altered by loss of the adrenals. Shortly before death, as is easily understood, the pressure fell. The blood pressure charts show no essential difference between adrenalectomized and non-adrenalectomized animals. The full paper was sent to the Journal of Metabolic Research in March, 1925. A similar result was obtained in dogs.

A number of observers state that they have found evidence that the epinephrin output can be increased experimentally by stimulation of afferent nerves and by total asphyxia. These statements, in our opinion, are based upon the misinterpretation of results obtained usually by inadequate methods. Our own quantitative results, while indicating quite clearly an augmentation by stimulation of the splanchnic nerves and by certain drugs, have revealed no increase, within the limits of accuracy of the method, either by sciatic or brachial stimulation or by general asphyxia. We may deal with this matter later in a separate paper but content ourselves here by putting the question: Even if it were

proved that such increases could be brought about, what physiological significance could they have, since suppression of the epinephrin output produces no recognizable change in the animal?

The details of the symptoms, *post mortem* appearances, etc., can only be dealt with satisfactorily by giving extracts from some of the protocols.

Dog 988, 1-20-25, right adrenal completely removed; 2-9-25, left adrenal completely removed at 2:00 p.m.; 2-10, blood in stools, diarrhea (about noon and later some more), took milk and biscuit; 2-11, no diarrhea, took rabbit and biscuit; 2-12, solid stools with blood stains, active and eating well; 2-15, excellent condition, eating well, feces normal. 2-16, strong and active like a normal dog, eating well (11:00 a.m.). At 4:00 p.m. blood-stained stools, dog lying on his side, refused food, came out of cage and walked quite steadily about the room. At 7:00 p.m. a quantity of practically pure blood on floor (from bowel), vomited several times (mucus), refused all food but walks around. 2-17, at 12:30 a.m., found considerable quantity of blood with a fecal odor; died between 2:30 and 3:00 p.m., bloody fluid was coming from anus and from mouth and nose.

The autopsy, made at once, showed the mucosa of the whole alimentary canal from cardiac orifice of stomach to anus, including the cecum and its process, to be tremendously congested and the seat of marked hemorrhages. Numerous blood clots are present in the lumen in the reddish liquid. There is bile everywhere as well as blood. Blood- and bile-containing liquid in mouth has obviously come from the stomach. Wound in left lumbar region well healed.

This animal illustrates a fairly common type but differs from the most common type in the prominence and early onset of blood in the stools. Possibly as much as 100 cc. of blood and bloody fluid was passed altogether from the bowel in life. Such a large amount of blood is quite unusual.

Dog 880. A wire-haired fox terrier, partly bull. Used for a control on the effect of intravenous injections of Ringer-dextrose solution (daily injections for about 2 weeks). Then after an interval of 2 to 3 weeks without injections, 1-7-25, right adrenal was excised; 1-14, developed spasticity and symptoms of "mad dog," isolated for a few days. Recovery seemed complete on 1-19-25, when left adrenal was removed at 2:15 p.m.; 1-20, bandage removed; ate a good meal; 1-21, active, ate well, developing spastic gait (trot-step with fore-legs). In the next 2 days the spasticity improved, appetite continued very fair; 1-25 to 1-30, good health, appetite good, dog active and happy, spasticity gone but it was noted again on 1-30; 2-1, eats less, spasticity present but not so marked, animal listless; 2-2, no appetite now, very asthenic, hardly able to stand, sleeps most of the time; 2-3, 8:00 a.m., very asthenic and lethargic; about 10:00 a.m. died without convulsions, weight 10.4 kgm.

Autopsy at once. Stomach, mucosa congested and small hemorrhages (erosions). Upper end of colon hemorrhagic, and blood in the contents. Some blood in contents of small intestine. Pancreas congested, also kidney and liver. This dog with its spastic symptoms and "mad" behavior developing after removal of one adrenal, the spasticity recurring from time to time after removal of the second gland, is not a common type. It is probable that in this case the symptoms were not connected with the adrenal deficiency. The period of survival was the longest in the whole series of control animals.

Nervous symptoms including hallucinations and dementia may occasionally develop in dogs, in a large stock, possibly sometimes associated with worms. We think, however, it is certain that such symptoms do occur in some cases as a result of adrenal deficiency. There is of course some variety in the symptoms, the most common form being a sudden "mad" attack, with loud yelling (and barking), the animal rushing around in its cage, apparently enraged or terrified by some imagined object. The eyes are staring, but the animal apparently does not see or recognize objects in its way. Outside of the cage it will rush across the room and bump into the wall. The animal seems to suffer from hallucinations. There may be only one attack but usually when the symptom occurs there are several or many in the course of the case. Among the 73 dogs in tables 1 and 2 there were at least 12 which showed this symptom in some degree, about a sixth of the whole number (viz., 986, 987, 1015, 1016, 1150, 1160, 1161, 1171, 1180, 1181, 1188, 1189), possibly more. In the other features of the symptomatology these dogs differed among themselves like the other dogs, e.g., in keeness of appetite and length of the period of good health. A few protocols are given to illustrate this group.

Dog 986. 1–16–25, right adrenal removed; 1–28, left adrenal removed; recovery excellent, appetite good, running around; 2–1, anorexia marked, previous day ate little, and nothing henceforth. 2–2, this forenoon, as dog 987 began to yell, 986 joined in, as if in sympathy. Later it was seen that he had the same symptoms as 987. At 7:45 p.m., he came off his bed, drank water, then vomited colorless liquid, stuck his head into a corner as if in slight spasm.

2–3, very asthenic, marked increase of reflex excitability, yells at times as if alarmed, and falls over when attempting to stand. At 3:00 p.m. yells more frequently, pupils dilated, cannot stand. At 7:00 p.m., conscious, lying on side, quite unable to rise. Increased reflex excitability. Legs (hind and fore) are in extension always and will not stay flexed when put in the flexed position. Responds by jerk of whole body and increased extension of the limbs to tactile stimulation anywhere and to loud sounds. At 8:30 p.m. reflex excitability is much diminished. Legs no longer in extension, will remain flexed when put in that position. Dog is comatose. Died at 9:00 p.m.

The autopsy, done at once, showed congestion and hemorrhage in stomach, small intestine, including the duodenum, blood in the contents, and congestion of the pancreas.

Dog 987, after recent parturition. 1–19–25, right adrenal excised; 1–28, left adrenal excised at 3:30 p.m.; good post-operative recovery, active and ate well.

2–2, active and strong up to this forenoon. Ate well till yesterday. This forenoon, symptoms as of great alarm began, yelling and dilatation of the pupils (they react to light), increased reflex activity and convulsions as in tetanus. This continued all day (with 2 convulsions). In the evening twitching of the head developed, movements sideways and forward-backward. At 7:45 p.m., breathing deeply, conscious, lying on side, yelling from time to time. At 8:15 p.m., still conscious but unable to change position. The whole body, except hind legs, jerks when any part, including the hind legs, is touched, reflex excitability markedly increased. Loud sounds cause similar jerking. Marked lid reflexes. No yelling now. At 10:00 p.m., conscious, passed semi-liquid stools. At midnight still alive.

2–3, found dead in morning. *Post mortem* appearances much the same as in 986. Gastric mucosa very much congested.

Dog 1161, mongrel, part collie. 12–21–25, right adrenal removed. The gland was deep and high under the liver and there was considerable trauma, the adrenal being crushed, but it was completely removed. The peritoneum was punctured. Such and similar accidents will occur, but they were not frequent. Post-operative recovery excellent. 1–13–26, left adrenal excised, excellent post-operative recovery. 1–14, condition very good; 1–15, active, eating well. At 11:30 a.m. had a violent barking fit lasting about 10 minutes; after it was over she wagged tail, seemed perfectly normal and ate a good meal of meat, etc. At 3:30 p.m. a yelling fit for a few minutes and then became normal; 1–16, active and eating well. 1–17, 1:45 p.m., took biscuit and most of her bread and milk, but is lying rather torpid on floor. At 10:00 p.m., lying on floor but promptly got up to eat a biscuit. Up to 1–19 unchanged, took biscuit and milk. At 2:00 p.m. refused to come out of cage. Door left open, and she suddenly rushed out and ran around the room in a crazy fit, was only got back with difficulty.

1–20, 12:00 m., refused biscuit. At 3:00 p.m. and 8:30 p.m. unchanged, lying on bed. At 9:30 p.m., lying dead on floor.

Autopsy at once. Pancreas greatly congested. Stomach considerably congested, as also colon and rectum. Some worms in small intestine and stomach. Contents consist of bile, no blood. Liver and kidneys somewhat congested, spleen not.

Among the most interesting of the cases in which marked nervous symptoms appeared were two Airedale pups of the same litter, about 2 months old. This is the only opportunity we had of removing the adrenals from dogs of the same litter. They were both males, so that the material was as nearly similar as it is possible to obtain. The protocols show that the symptoms (including the time of onset) and *post mortem* appearances were almost identical. The period of survival was relatively short, and not very different in the two animals. The important question of the cause of the variations in different animals, and whether they are narrower in animals of the same litter cannot of course be settled by one case, and the findings are simply given as having a possible bearing upon this question. It should be noted (see table 1) that in dog 1015 the right adrenal which was removed 14 days after the left was considerably heavier (compensatory hypertrophy?), although there was no change in body weight.

Dog 1015. Airedale pup. Only milk, bread and biscuit diet; 4–6–25, excised left adrenal (time of operation 15 minutes); 4–20, excised right adrenal at 1:30 p.m.; excellent post-operative recovery.

4–21, 4:00 p.m. developed "mad" symptoms, which lasted 10 to 15 minutes. 7:30 p.m., when light was turned on in the room the pup suddenly began to scream and bark, running around in the cage. This started off the smaller pup (1016). Both were wild-eyed. At 9:30 p.m. there was another "mad" attack on observer going into the room. Dog 1016 followed suit. But while 1015 took a biscuit practically in the middle of an attack and desired more, 1016 paid no attention to biscuit.

4–22, 12:15 a.m., the worst fit of all, both dogs running around in their cages,

howling and trying to climb out, hallucinations, eyes starting as in terror. At 6:30 a.m., and 7:15 a.m. similar performances, deafening yells, dog 1016 generally leading off and 1015 joining in. This continued at intervals all day up to 11:00 p.m. But 1015 eats sufficiently well, while 1016 refuses food.

4-23, 7:00 a.m., is hungry and eats biscuit and milk readily, while 1016 at first refused but finally took a little. "Mad" attacks continue, especially when started by 1016. Later on would not eat.

4-24, 7:20 a.m., took some milk but refused biscuit. Has not been crazy since yesterday. At 3:00 p.m., very weak, but can be roused. At 6:00 p.m. semi-comatose, and unchanged at 11:00 p.m.

4-25, 6:45 a.m., same condition, deep, slow, labored breathing. 7:45 a.m., convulsions, treading movements as he lies on side. Died between 8:30 and 9:00 a.m.

Autopsy at once. Pancreas moderately congested. Stomach congested except a small part at pyloric end. No hemorrhages. Contents practically pure bile, no blood. No special congestion in small intestine, which contains bile, especially duodenum. Congestion and hemorrhage at lower end of large intestine. Wound clean. Dura mater seems congested over central portion of cerebral hemispheres.

Dog 1016, pup, brother of 1015. The protocol has been partly given with that of 1015. 4-6-25, excised right adrenal (total time 12 to 15 minutes). Quick recovery (in 20 to 30 minutes); 4-20, excised left adrenal at 2:00 p.m., excellent recovery; 4-21, eating biscuit and milk well. At 4:00 p.m. went into a mad fit, yelling and snapping, etc. This lasted 10 to 15 minutes and started off dog 1015. 4-22, took some milk in morning, but nothing more today. Mad spells during the day; 4-23, no food taken, mad spells continue. Gave dose of "worm medicine" and castor oil, but no effect. Alive at 11:00 p.m. but in poor condition. 4-24, 7:00 a.m., found dead.

Autopsy. Pancreas moderately congested. Stomach contained a round worm and brownish, bilious liquid (15 cc.). No congestion anywhere in alimentary canal. Contents have a fecal odor, small amount of blood pigment (spectroscope). Elsewhere in gastro-intestinal tract only bile. Wound clean.

The next protocol (991) illustrates a very small group of cases, dying as is believed from adrenal insufficiency, in which no food was taken after removal of the second adrenal. A feature common to these cases is that the total period of survival is decidedly below the average. The pathological changes in the gastro-intestinal tract and pancreas may be well marked (as in 1031, table 2), or less marked than usual (as in 991, table 1). In 991, greater difficulty than usual was experienced in taking out the second adrenal (left), but it is not known whether this had anything to do with the continued refusal of food and the short period of survival.

Dog 991. 1-30-25, excised right adrenal; 2-11, excised left adrenal at 2:00 p.m., there was a small hemorrhage in removing the gland and more trauma than usual; 2-12, refused food, lies about apathetically but able to walk all right; 2-13, somewhat more alert, took no food, took water and vomited mucous fluid: 2:00 p.m., small amount of whitish vomit (mucus), no bile, strongly acid. 2-15, 10:00 a.m., refused food (rabbit), sleeps much. No change in this regard since last observation. At 2:00 p.m. vomited a little liquid with mucus, neutral. Dog is not specially weak but lies curled up on bed. Died at 4:45 p.m.

Autopsy (at once). Mucosa of ileum congested; also mucosa of colon (some

hemorrhages). Jejunum, ileum and colon contained bile and some blood. Nothing abnormal in rest of gastro-intestinal tract. No worms. Pancreas moderately congested. Kidneys and liver much congested. Stomach contents strongly acid, no blood, Gmelin's test negative. Wound very well healed. No infection.

In the most common type of case nervous symptoms are inconspicuous. Apart perhaps from nausea and vomiting in some of the cases, the animal appears to suffer little, if at all, and the exitus, preceded by a variable period of coma, is quiet. The most characteristic symptoms have been described earlier in the paper. More than half the animals in tables 1 and 2, probably considerably more, belong to this group (including dogs 985, 992, 993, 995, 998, 999, 1001, 1008, 1009, 1018, 1019, 1020, 1022, 1025, 1027, 1033, 1037, 1038, 1126, 1136, 1138, 1145, 1146, 1148, 1149, 1151, 1154, 1158, 1163, 1182, 1186, 1197). Some of these will be referred to in paper II. Here only a few representative protocols can be given.

Dog 985, 1-16-25, excised right adrenal. Body weight 10.6 kgm.; 1-26, excised left adrenal at 3:20 p.m. Body weight 10.0 kgm. Good post-operative recovery. Remained well and taking food normally till 2-3-25, when he took less than usual; 2-4-25, refused food; the last food taken was meat at 7:45 p.m. on 2-3-25. Body weight 9.5 kgm. Has been rather listless for 2 or 3 days, but showed no weakness; 2-5-25, died during the night.

Autopsy. Liver, kidneys and pancreas congested. Stomach, bile, no blood in contents, same for duodenum. Ileum, lower colon and rectum congested, with hemorrhages. Small blood clots in contents. In rectum the blood is fresh; in ileum many erythrocytes recognisable but altered. Blood pigment recognised by spectroscope in large amount (hemochromogen after KOH and $(NH_4)_2S$).

Dog 1001, young fox terrier. 2-20-25, weight 8.3 kgm., excised right adrenal; 3-9-25. Weight 7.35 kgm. Excised left adrenal at 2:00 p.m. 3-10, perfect post-operative recovery, active and lively and ate well. Weight 7.1 kgm.; 3-12, like a normal dog; 3-13, played (roughly) with other dogs, health and appetite good, and continued so till 3-16, when she refused food for the first time. Now lies about most of the time but plays when invited. Did not eat after this. 3-17, 7:00 a.m., less playful, refused milk, and has not eaten the biscuit she got last night; does not care to run about. At 10:30 a.m. temperature 38.1°C.; pulse 152; respiration 22. Peg leg walk (hind legs). Weight 7.0 kgm. At 2:00 p.m. washed stomach; green washings returned at first, showing that bile is now in stomach, then clear; followed by castor oil [℥ii] and later 8 grams dextrose in 100 cc. water. Retained 20 to 30 minutes, then vomited large amount of watery green liquid (acid to litmus and giving good test for sugar).

3-18, 1:30 a.m., unable to stand, lying half asleep. 7:00 a.m., very weak, comatose, has vomited liquid with blood pigment in it. 8:10 a.m. died.

Autopsy at once. Stomach, mucosa very hemorrhagic and clots on it. Duodenum, only pyloric end showed much congestion. Jejunum and ileum (except lower part of latter) much congested and hemorrhagic with clots. Bloody contents in jejunum. Pancreas congested. No worms.

Dog 1148, part bull-terrier. 12-1-25, excised right adrenal, operation took 12 minutes. Body-weight 11.5 kgm. 12-14, body weight 11.55 kgm.; 12-24, excised left adrenal at 1:25 p.m. in 10 minutes. Body weight 11.4 kgm., excellent post-operative recovery. 1-1-26, condition to date excellent. Demonstrated at meet-

ing of Federated Societies 12–29–25. Eating very well, very active and vigorous. 1–2–26, unchanged, eating well; 1–3–26, 1:00 p.m., same excellent condition as yesterday, but began to refuse food, and thus continued.

1–4–26, refusing food, "stretches" occasionally and when walking the back seems beginning to sag "camel-like" at times. No wobble but lies about in cage more than before. Has had cough and nasal discharge for 3 or 4 days, discharge worse today. 7:30 p.m., poor condition, bilious vomiting with mucous flakes in vomit, alkaline. Lying on bed, does not lift head but wags tail. Still tries to catch flies; 11:00 p.m., lying on floor, completely comatose. Has passed a semi-liquid stool and is lying in it; 1–5–26, 1:30 a.m. died.

Autopsy at once. Stomach mucosa congested, except at pylorus, with some hemorrhages. Stomach and duodenum contained bile. Congestion increases from upper part of jejunum to and including upper part of ileum. Contents bloody. No worms. Rectum much congested. Upper part of colon not congested. Pancreas decidedly congested. Lymph glands (pancreas Aselli) congested. Omentum much congested. Liver and kidneys congested, spleen not specially.

Dog 1158, fox terrier, 12–19–25, excised right adrenal. 12–26, excised left adrenal at 11:30 a.m., excellent post-operative recovery. 1–1–26, condition very good up to date, active and eating well. Demonstrated to Federation 12–29; was observed several times a day up to 1–6–26, when he was still in good condition. He ate a good meal of meat and tried to start a fight repeatedly with a new dog; 1–7–26, unchanged, eating well, tried to fight his neighbor; 1–8–26, lying on bed, unwilling to come out of cage, somnolent. Stands up all right when coaxed into that position. No longer playful. Pulse good, 122 a minute. At 11:30 a.m. refused all food. About 9:00 a.m. he picked a quarrel with another dog and barked violently at him. But he is now so suddenly changed that he pays no attention to the barking of this dog. Walked (with some wobble) about the room, but is apathetic. At 12:00 m., vomited bile-stained liquid. 3:45 p.m., lying on bed listlessly. Heart weaker and more rapid, skin beginning to get cyanotic. When walking head hangs down, also tail; he looks depressed. At 8:15 p.m. ate a small piece of ham (for him a dainty). Quite wobbly on his feet.

1–9–26, 11:15 a.m., pulse 120 (easily felt in femoral). Weak on feet but can walk. Spreads the legs. Occasional jerks, ears and shaved skin cool. Defecated and urinated, blood in feces, urine acid to litmus. 5:30 p.m., spasmodic jerking becoming more marked. Weakness increasing each hour. Can still walk across the room, although very wobbly. Rectal temperature 37°C.; 8:15 p.m., can scarcely stand, props himself against a cage, or totters a step or two. Jerking of head occasionally. Refused ham although he recognized and sniffed at it. 9:00 p.m. asleep. At 9:30 p.m. walked a few steps with ataxic gait. Pulse 132. Conscious. 10:00 p.m., tetanic convulsion. Then coma and violent jerking of head, jaw and legs, then chronic spasms. Two more tetanic convulsions; died at 10:40 p.m.

Autopsy (at once). Stomach, mucosa congested, but not intensely (two quite small erosions), contains a few cubic centimeters of bile. Liver, kidneys and pancreas much congested; spleen congested (blue). Duodenum and upper part of jejunum much congested with blood and bile in lumen. Same for ileum. Many small blood clots. Same for colon and rectum.

For shorter periods of survival, about a week, falling in this group the protocols of dogs 995 and 999 will suffice.

Dog 995, spaniel, 2–4–25, excised right adrenal, slight hemorrhage from a lumbar vein and more trauma than usual. Body weight 8.0 kgm. 3–2–25, excised left adrenal at 2:30 p.m. Body weight 8.7 kgm.; 3–3, refused food but seems in good condition; 3–4 and 3–5, active and eating well; 3–6, vomited all his forenoon meal; 3–7, good condition, but appetite rather poor. At 10:00 p.m. found solid and semi-solid feces with some blood.

3–8, running about actively but taking no food; vomits (bile), (8:30 a.m.), bright greenish-yellow, strongly acid to litmus. Dog ran along corridor; interested in white rats. No weakness whatever. Seems entirely normal. Took a very little milk. Laps water. Refused food. More stools were passed throughout the day, solid or liquid, containing blood (tarry), in form of small flakes or clots. Total amount of blood passed was not large. Bile pigment in feces also (Gmelin). More vomiting of bilious character throughout the day, reaction weakly acid to litmus. Complete anorexia, but animal is not weak, and quite willing to take a walk although he lies about a great deal. At 7:30 p.m. vomit containing 4 or 5 round worms, two of them 6 to 8 inches long (alive). Another small stool with considerable blood. At 9:15 p.m., vomit same as before but no worms, strongly acid to litmus, little proteolytic power (Mett's tubes) with or without HCl; 3–9–25, 12:15 a.m., more vomit, same as before. At 7:30 a.m. and 11:30 a.m. refused milk and meat, but took a walk. Defecated. No blood in stool (spectroscope). Less active today. Complete anorexia.

3–10, 7:00 a.m., has vomited several times in the night, once with bile. Saw him vomit liquid free from bile (strongly acid). Much nauseated. The vomit (free from bile) gave a strong biuret reaction. Same for subsequent vomits. Bloody stool. He became rapidly worse this morning. At 7:00 a.m. he was standing up and wagged tail. Died at 9:10 a.m.

Autopsy (at once). Gastro-intestinal mucosa greatly congested from cardiac end of stomach to anus, including cecum. Pancreas moderately congested. Some blood pigment in the bile-containing liquid in stomach (hemochromogen test). Plenty of blood and small blood clots in lumen of intestines (small and large), and in the cecum. Kidneys quite congested, spleen moderately.

Bladder contains 55 cc. urine, bright yellow, but not containing bile (Gmelin and Hay's tests negative). Urine strongly acid to litmus, no coagulable protein or sugar. Defibrinated blood had only 15 per cent of serum. Conductivity for blood, K (25°) $\times 10^4 = 9.1$; for serum 101.2.

Dog 999, mongrel pup, 2–13–25, excised right adrenal. Body weight 8.0 kgm.; 3–2–25, excised left adrenal at 2:00 p.m. (total time of operation 20 minutes). Body weight 7.5 kgm.; 3–4, very active, eats ravenously, slight snuffles; 3–5, 12:45 p.m. vomited food recently eaten and much yellowish liquid (Gmelin's test negative), strongly acid; 3–6, very active, eating voraciously. Passed a round worm (2½ to 3 inches long) from anus, 3:00 p.m.; 3–8, active but not eating as usual since yesterday. Ate a little today. At 8:30 a.m. coughing. Below par. Took 2 or 3 laps of milk, nothing else. Drank much water. No desire to run around. At 8:00 p.m. came out of cage, took water.

3–9, 12:15 a.m., got off bed to vomit, greenish-yellow, frothy liquid, precisely like vomit of dog 995 (strongly acid). Defecated, some flakes of blood in stool. Walks around cage and takes much water. At 3:00 a.m. unchanged. Poor condition. At 7:30 a.m., much the same, refused milk. Came out of cage, but staggered and immediately lay down by the wall. (It is common to see dogs at this stage get close into a corner and lie down.) Seems nauseated; 11:30 a.m., vomited 30 or 40 cc. yellowish-green liquid (strongly acid). This did not digest protein (Mett's tube) in 24 hours in thermostat. Dog very weak, fell down in vomiting and had a

slight spasm. Laps water afterwards, but is too weak to take all he wants. Pads of feet cold. Earlier in the forenoon he ran around as if "crazy," giving an occasional whine. Total anorexia. In afternoon had short convulsion. At 1:30 p.m., very weak, pulse 66 (regular), respiration 21 a minute. At 2:45 p.m. a semi-solid stool passed, containing a little blood. At 3:00 p.m. he is lying on side making vain vomiting efforts. Pulse 66 (fairly regular), respiration not slow. At last vomited some colorless frothy liquid not containing bile and strongly acid to litmus. At 4:45 p.m. same position. Reflex excitability somewhat increased. Pulse 60 (not weak), respiration about the same as before. At 5:25 p.m. respiration 32 a minute. Pulse feebler, not rapid. Convulsive chewing movements. Died at 6:30 p.m.

Autopsy (at once). Stomach, lower part of jejunum and ileum are decidedly congested, also pancreas; same for lower end of colon and rectum to anus. Blood-containing contents in the corresponding portions of the tube; skin around anus smeared with blood. Conductivity of serum K (25°) $\times 10^4$, 111.0.

Discussion. As regards discussion of the relation of the symptoms to the pathological appearances something will be said in paper II after the results of the blood studies have been analyzed. It will suffice to point out here that in a very considerable number of the cases the condition of the gastro-intestinal mucosa is of itself assuredly incompatible with health and sufficient to account for the quickly fatal result. The question of the causation of the symptoms and of death in such cases would be much the same as the question of the causation of the gastro-intestinal changes. Probably other organs, e.g., the heart, are being intoxicated at the same time, perhaps as a consequence of the crippling of the alimentary mucosa. The characteristic anorexia is easy enough to account for in such cases. We do not, however, know the time of onset of the pathological changes in the mucosa. A clue is given in not a few cases by the passing of bloody feces. We have also occasionally given castor oil to clean out the bowel in order to determine whether blood was present at a given time. For example, in dog 1009 (table 2), food was first refused about $3\frac{1}{2}$ to 4 days after removal of the second adrenal and the animal seemed to be getting less vigorous. The scanty feces were dark. Castor oil was administered, and dark feces were passed with many spots of fresh blood, followed by liquid stools with blood and bile. We concluded that at this time there was some blood in the lumen of the bowel. It has already been stated that the quantity of blood passed from the bowel is no exact indication of the condition which will be found *post mortem*. We have not hitherto had any surplus of adrenalectomized dogs, else the direct method of settling the relations between the symptoms and the gastro-intestinal changes by sacrificing some animals for autopsy could have been adopted.

The apparently close relationship between the presence of the adrenals and the continuance of normal function in the gastro-intestinal tract suggests the study of possible changes in the digestive secretions and the

motility of the tube by means of fistulae in adrenalectomized dogs. It was thought also that inspection of the mucosa from time to time during development of the symptoms might throw light upon the questions under discussion. Much more work is necessary. A gastric fistula was made on 3 dogs after recovery from the first adrenalectomy. After 11 days to about a month (in 2 of the dogs, 1126 and 1136), during which observations of juice, movements, vascularity, etc., of the stomach were carried on, the second adrenal was excised. Recovery was good, but only 2 of the dogs lived as long as 4½ days. It is not known whether the gastric fistula had any influence upon the survival period. Observations made at intervals throughout the period of survival did not reveal any striking change in the movements of the stomach or the properties of the juice. Meat put into the stomach was digested. In dog 1126, almost 3 days after the second adrenalectomy, when serious nervous symptoms ("mad" fits) had developed, food (meat, broth and milk) was taken by mouth. The juice flowed as freely as before the adrenalectomy; the free KCl was 106, the total acidity 114. Peristalsis was vigorous. Meat placed in the stomach was digested. The mucosa was decidedly hyperemic. Half a day before death the gastric juice still flowed well and plenty of HCl was secreted (free acidity 104, total acidity 112). Mett's tubes showed good digestion by juice collected the 2nd day, the 3rd day and the 4th day after the second adrenalectomy. The mucosa was covered with mucus, was less red than the day before, and the peristalsis was weaker. The animal took broth and also water by the mouth and meat was given by the fistula. She had appetite for food. At autopsy the stomach and duodenum were not congested, the lower part of the jejunum and the ileum were congested and had blood in the contents. This was also true of the large bowel. The pancreas and liver were much congested. In dog 1136 the findings were much the same as regards the acidity and proteolytic power of the gastric juice and the movements. It can often be seen in dogs dying after adrenalectomy that the vomit remains decidedly acid to litmus to the end, indicating a continued secretion of HCl, although nothing has been eaten for days. If the vomit is bilious it may, of course, be only weakly acid or neutral or weakly alkaline. It may then be distinctly putrid or even fecal in odor.

An interesting additional observation was made in dog 1136, which has a bearing on the possible mechanism of intravenous injection of salt solutions in prolonging life in adrenalectomized animals (paper IV). Four days after the second adrenalectomy, when he was refusing food (since the previous day), very weak and scarcely able to walk a few steps, the gastric juice flowed very slowly (only somewhat more than 3 cc. in 20 minutes; total acid 35, free HCl 32 to 33). The blood flow was also very slow, as seen in withdrawing a blood sample. An injection of 900

cc. Ringer's solution with 5 grams dextrose was now made into a vein (much more slowly than usual). The gastric mucosa gradually became paler, and at the end of the injection it was much paler than before. The flow of gastric juice was greatly quickened. Two specimens were collected, one in the early part of the injection (11.5 cc. in 15 minutes; total acid 105, free HCl 106), and another in the latter part of the injection (over 11.5 cc. in 15 minutes; total acid 108, free HCl 104). The mucus content and viscosity of the last two specimens were much less than in the specimen taken before injection, and in the last specimen least of all. It is clear that even at this stage when the animal was moribund the injection induced a copious secretion of HCl and water, the total HCl per minute being much increased. It is suggested that salt solution injections may act in staving off the intoxication, or in causing temporary benefit after it has appeared, by washing out the poison, possibly by destroying it. If the gastro-intestinal mucosa is one of the channels, perhaps the principal one, through which the toxic substances are got rid of, it is easy to see that it might itself be crippled sooner or later. This would account for the development of the fatal symptoms at different periods after the second adrenalectomy and for the characteristic changes found *post mortem* in the alimentary canal.

It is perhaps only putting the matter in different words to say that in the absence of a cortical hormone a breakdown centering about the alimentary canal occurs sooner or later, being seldom postponed beyond a week. Whether we think of the hypothetical hormone as a substance with a specific relation to the gastro-intestinal tube and indispensable to the normal continuance of its functions or not, it is clear enough that in the absence of the cortex a serious failure or derangement does occur, probably with a tendency to reversed peristalsis and certainly with displacement of contents into abnormal situations, e.g., bile into the stomach. The connection of this derangement with the chief symptoms seems fairly direct. Although the dogs stop eating entirely it may be noted that they never die of lack of food, for death occurs much too soon. At autopsy plenty of fat has invariably been found in the usual situations. At present it cannot be stated that other eliminating organs, e.g., the kidney, do not share in the breakdown, although there is no evidence that the kidney is concerned.

SUMMARY

As a basis of comparison with dogs subjected to treatment, or pregnant, tables are given comprising the results of observations on 34 male dogs and 39 non-pregnant females. Another non-pregnant female has been added since, making a total of 74 controls. Pregnant animals are excluded from this control series because, as we have shown, the period of survival is mark-

edly lengthened by pregnancy. The adrenals were removed in a two-stage operation, with an interval varying from about a week to about 6 months. An interval of a week was found to give as good results as a longer one. The majority of the dogs (about two-thirds) lived 4 or 5 to 8 or 9 days after the second adrenalectomy. A fair number (about one-tenth) lived 10 to 12 days. Two dogs lived into the 15th day. About one-sixth of the animals survived 4 days or less.

The animals recover rapidly from the second operation. It is a general rule that with the shorter times of survival the period of normal health and good appetite is relatively less than with the longer survival periods. It is a consequence of this that the time for which the dog still lives after the anorexia, the most characteristic and constant symptom, has declared itself is not very different in the great majority of the animals, whether the total survival period be long or short. Most of the dogs died in less than 3 days after definitely refusing food. Other symptoms (bilious vomiting, nervous symptoms, asthenia, etc.) are described.

The pathological changes found at autopsy, especially in the gastro-intestinal tube (congestion and hemorrhage in the mucosa, blood in the lumen) and in the pancreas are indicated in the tables, and details are given in the protocols cited.

Blood examinations of various kinds were made on about 35 dogs. For convenience these are included in a separate paper (paper II), but the two papers constitute a single study. The possible relations of the blood changes to the clinical and pathological picture will be discussed in paper II.

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STUDIES ON ADRENAL INSUFFICIENCY IN DOGS

II. Blood Studies in Control Animals Not Subjected to Treatment

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This constitutes a part of paper I, and is separated merely for convenience. When the clinical picture described in that paper is studied the question inevitably presents itself whether at the time of appearance of the symptoms which announce that the period of good health is over or is soon to terminate, any physical or chemical changes can be detected in the blood. As already remarked the symptoms, when fully developed, and the pathological findings suggest a profound and steadily advancing intoxication as the immediate cause of the derangement. The poison or poisons may be supposed to go on being produced after the second adrenalectomy but to be eliminated or neutralised for a longer or shorter time till at last the neutralising or eliminating mechanism, whatever it may be, breaks down or uses up a preformed store of irreplaceable material. If it is the absence of a cortical hormone which is responsible for the derangement it makes no difference. Only it must now be assumed that some stock of this substance or of material which can be changed into it even after removal of the cortex keeps the animal going and staves off the intoxication for a variable period till the stock becomes exhausted.

If toxic substances, accumulating in the blood or tissues, are the essential factors it is not to be expected that their detection and identification will prove easy. Attempts to obtain evidence of the existence of such poisons by biological tests were made long ago by Abelous and Langlois (1892, 1893) and others. It cannot be said that anything has been definitely ascertained. Our purpose was limited to the estimation of such determinable quantities as the content of sugar, non-protein N, urea N, amino-acid N, uric acid, preformed and total creatinine, and Cl. Some estimations of serum calcium and serum proteins were made, and in many animals the conductivity of the blood and serum, the relative volume of erythrocytes and serum, the specific gravity, and the Hb percentage were determined with counts of erythrocytes and leucocytes. It was obvious that if any

quantitative changes occurred either when the acute symptoms came on or possibly preceding them, their occurrence and development would be much better followed in dogs surviving a relatively long time than in practically moribund animals. Our opinion is that blood studies of the kind mentioned are of no value unless the best technique is followed in preparing the adrenalectomized animals.

Non-protein nitrogen, etc. The results, on 12 dogs, of the estimations of non-protein N, urea N, uric acid, preformed and total creatinine, amino-acid N, Cl (expressed as NaCl) and dextrose are embodied in table 1, the dextrose as grams per 100 cc. blood, all the other quantities as milligrams per 100 cc. of blood. The last meal was almost always about noon on the day previous to the abstraction of the blood. The analyses of blood (and urine) were performed chiefly by the methods described by Folin in his manual. Uric acid was determined by Benedict's method and calcium by Kramer and Tisdall's method as modified by Collip. It may prevent occasional misapprehension to state that the dogs in table 1 were all control adrenalectomised dogs, not subjected to treatment. They are all included in tables 1 and 2, paper I, where data on survival period, etc., are given.

Some of the points which seem to be established by the data in table 1 will now be taken up. It is impossible to include in the table sufficient information as to the condition of the animal at the time the various blood samples were drawn. Yet this is indispensable if the chemical findings are to be related to the clinical picture. Extracts from the protocols must therefore be reproduced. As regards the non-protein nitrogen, a great increase (to 5 or 6, or even as much as 7 or 8 times the initial value) takes place in the last days of life when the animal is no longer eating. This is shown by all the dogs in the table. It should be explained that in dog 1186, the specimen on June 20, in which the non-protein nitrogen was about 4 times the initial amount, was taken 24 hours after the last meal, after which the animal refused all food. The chief part of the increase in the non-protein nitrogen is due to the increase in urea. When the increase in non-protein nitrogen is great the increase in urea, although marked, is not generally proportionate, the difference being made up mainly, at any rate, by a corresponding increase in the "undetermined fraction." There are a few exceptions to this rule, e.g., in dog 1189, blood sample of April 6, and dog 1196 both blood samples of June 26. In these cases practically the whole increase in the non-protein nitrogen is due to the urea. Not infrequently some increase is seen in the non-protein nitrogen before the dog has stopped eating and while he is still in good health. Sometimes this is due entirely to the increase in urea, sometimes, in addition, to an increase in the undetermined fraction, e.g., in dog 1146, May 25 and 26. As the protocol shows, on these two dates the dog was still eating well and was in good health. We do not see how the decided in-

crease (to twice the initial value) in non-protein and urea nitrogen in these blood samples can be described as other than prodromal with reference to the anorexia. In the next two samples there was, of course, a much greater increase. The same thing is seen in dog 1186 in the blood samples of June 16, and in dog 1182 in the sample of May 28. The protocols show that both animals were in excellent health on these dates. At no stage is there any decided increase in the uric acid or the amino-acid nitrogen. Occasionally the creatinine seems, in the terminal stages, to undergo some increase. Rather frequently the chlorine is apt to undergo some diminution in successive samples, especially when the animal has stopped eating and the non-protein nitrogen is increasing rapidly (see dogs 1149, 1186, 1195, 1197, 1200, 1204, table 1). That there is often some diminution in the chlorides in the last days of life is corroborated by the fact that at this time the electrical conductivity of the serum, a quantity which can be measured very accurately and which varies little under normal conditions, is also diminished. The possible significance of this will be alluded to later on. The dextrose is little if at all affected till near the end when it may be moderately diminished and even this not constantly. Thus in dog 1182 on May 20, the day before the second adrenalectomy it was 0.08; two days after the operation the same, then as low as 0.06 while the dog was still in good health; even on the day before death it did not go below 0.06 per cent. In dog 1146, the dextrose on the day before the second adrenalectomy was 0.07 per cent. Two days after the operation and for several days thereafter it was 0.08 per cent, and on the day of death 0.07 per cent. We find in these observations corroboration of our view, more than once expressed, that the supposed hypoglycemia after removal of the adrenals, commonly attributed to loss of the epinephrin secretion, does not exist; unless as a terminal phenomenon in dying animals.

The urine analyses, some of which are collected in table 2, are of much less significance than the blood analyses. They were not done on samples of the 24 hours' urine but merely on samples obtained by catheter, usually at the same time as the blood samples. The diet was not controlled, except that for the last days the animals would not eat and for 24 hours after the operation they were not allowed to eat. The only object of the urine examination was to look for gross departures from the normal. None was found, although more systematic work on the urine is necessary. Qualitative examinations of the urine were made in a great many animals without yielding evidence that the renal function was in any important way deranged. Not more than once or twice in all our animals, even during the last hours, or in the urine taken from the bladder after death, was coagulable protein found, and then only in small amount. Sugar was never found by the ordinary qualitative tests. In spite of the apparently great quantities of bile in the alimentary canal, and in abnormal

TABLE 1

Blood analy is

NUMBER OF DOG	BLOOD TAKEN, DATE	PREVIOUS MEAL	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE		AMINO ACID NITROGEN	UNDETERMINED NPN	DEXTROSE	Cl as NaCl
						Preformed	Total				
1146	5–20	Meat	33	16.7	1.0	0.9	2.2	10.0	5.2	0.07	536
	5–23	Meat	31.2	15.5	1.0	0.9	2.4	9.0	5.6	0.08	503
	5–25	Meat	58	27	1.1	0.9	2.4	12.7	17.1	0.08	510
	5–26*	Bread, broth	60	37.5	1.3	1.1	3.1	11.7	9.3	0.08	520
	5–27*	Meat	120	67	1.2	1.2	3.3	11.7	40	0.08	510
	5–28	Not eating	171	120	1.3	1.5	3.6	10.8	38	0.07	510
1149	6–7	Bread, milk	25.6	9.5	2.3	1.0	2.2	10.4	4.2	0.09	478
	6–12	Bread, milk	30	9.7	1.5	1.1	2.7	8.8	10.1	0.10	454
	6–14	Bread, milk	30.8	13.1	1.5	1.1	2.2	10.3	6.2	0.08	445
	6–16	Bread, meat	62.5	31.5	1.6	1.2	3.2	9.3	20.1	0.07	430
	6–17	Not eating	100	55	1.4	1.4	3.3	9.2	34.2	0.07	437
	6–18	Not eating	159.3	100	1.7	2.7	10.4	10.3	44	0.08	430
	6–18†	Not eating	216	118		3.2	6.8			0.09	410
1182	5–20	Meat	38	20	1.2	1.0	2.2	10.7	6.2	0.08	520
	5–23	Meat	30	12.5	1.1	0.9	2.7	10.9	5.3	0.08	478
	5–24	Bread, milk	30	13	1.2	1.1	2.4	10.0	5.8	0.07	478
	5–25	Meat	44	25	1.2	1.1	2.5	9.7	8.1	0.07	437
	5–26*	Bread, milk	43	24	1.3	1.0	2.8	11.7	6.0	0.06	445
	5–28	Meat	81	43	1.2	1.3	3.2	10.8	25.7	0.06	478
	5–29†	Meat	189	112.5	2.2	2.2	3.2	9.3	65.4	0.07	487
	5–31	Not eating	273	115.5	2.5	2.5	4.3	10.2	145	0.06	520
1186	6–7	Bread, milk	27	10.5	2.6	1.1	2.2	12.0	2.9	0.09	544
	6–12	Bread, milk	29	10.3	1.6	1.2	2.6	8.8	8.5	0.09	503

	6–14	Bread, milk	29.2	10.3	1.5	1.2	2.5	10.0	7.6	0.08	495
	6–16	Bread, milk	31.6	11.9	1.3	1.1	3.0	8.8	9.5	0.09	462
	6–17	Meat	48	29	1.4	1.1	2.5	9.3	8.4	0.08	445
	6–18	Bread, broth	38	18	1.7	1.1	3.2	8.8	9.6	0.09	478
	6–19	Rabbit	68	48		1.4	3.2			0.07	437
	6–20	Rabbit	102.5	74	1.6	1.9	4.3			0.08	430
1188	4–27	Meat	48	25.4	1.2	1.3	3.5	13.0	8.0		462
	4–30	Bread, milk	30	10.3	1.4	1.2	3.0	10.0	8.2		495
	5–2	Meat	44	20.7	1.3	1.0	3.3	14.7	7.1		454
	5–3	Bread, broth	34	17	1.4	1.0	2.3	10.0	5.8		470
	5–4	Not eating	60	33	1.5	1.0	2.3	11.4	14.3		478
	5–5	Not eating	133	86	1.9	2.5	6.3	15.5	28.8		495
1189	3–29	Meat	25	14	2.0	1.2	3.2	7.7	1.6		
	4–3	Bread, etc.	54	38	1.1	1.5	3.3	9.6	4.9		510
	4–6†	Not eating	156	132	1.3	2.0	4.4	10.7	11.4		470
	4–7	Not eating	167	120	2.8	2.0	5.0	10.1	34.3		470
1195	6–8	Meat	39	18	2.2	1.1	3.1	9.0	10.2	0.10	478
	6–21	Rabbit	36.6	20	1.7	1.0	2.7	12.0	3.1	0.08	462
	6–22	Not eating	94	57		1.5	4.3			0.09	437
1196	4–29	Meat	41	19	2.3	1.1	3.3	12.0	8.1		495
	6–8	Meat	39.6	14.3	2.8	1.1	3.1	9.6	13.7	0.10	478
	6–25	Not eating	31.6	14.3	1.7	1.0	2.7	8.4	7.4	0.08	445
	6–26	Not eating	46	31.5	1.8	1.0	3.0	11.7	1.2	0.07	445
	6–26†	Not eating	60	43	2.0	1.4	3.4	10.8	4.4	0.09	430
1197	4–29	Meat	39	21	1.7	1.0	3.1	10.8	5.6		520
	6–5	Not eating	49	22.3	3.4	1.0	2.4	8.8	16.0	0.06	470

TABLE 1—*Concluded*

NUMBER OF DOG	BLOOD TAKEN, DATE	PREVIOUS MEAL	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE		AMINO ACID NITROGEN	UNDETERMINED NPN	DEXTROSE	Cl AS NaCl
						Preformed	Total				
1200	4–23	Bread, milk	34	14	1.5	1.4	3.0	11.0	7.5		520
	6–9	Bread, milk	26	11.5	3.2	1.1	3.9	9.6	2.5	0.10	487
	6–21	Bread, milk	35	15.8	1.7	1.0	2.9	9.3	8.4	0.09	478
	6–23	Not eating	48	19	1.8	1.1	3.0	9.6	17.8	0.10	445
	6–23†	Not eating	54.6	33.6	2.1	1.3	3.3	11.6	7.6	0.11	420
	6–24	Not eating	69.3	45	2.0	1.2	3.7	10.4	12.0	0.09	420
	6–25	Not eating	116	69	1.9	1.4	3.5	9.3	35.1	0.08	410
	6–26	Not eating	192	105	2.1	2.3	8.0	12.7	70.8	0.08	445
1203	5–18	Meat	34.7	16	1.3	0.8	2.0	10.7	6.9	0.09	520
	6–5	Not eating	50	33.3	2.5	1.1	2.5	8.9	6.1	0.06	520
1204	6–9	Bread, milk	30	12.8	2.8	1.2	4.2	8.8	6.1	0.09	544
	6–21	Bread, milk	35	12	1.4	1.0	2.9	8.0	13.6	0.07	495
	6–22	Rabbit, etc.	78	55	1.3	1.1	2.6	8.0	13.7	0.08	487
	6–23	Rabbit	82	50	1.4	1.3	3.3	9.1	21.3	0.09	503
	6–23†	Not eating	75	54	1.4	1.3	3.4	11.3	8.1	0.09	495
	6–24	Not eating	91	62	1.5	1.2	3.7	8.8	18.5	0.08	503
	6–25	Not eating	180	96	1.4	1.7	5.4	11.0	70.7	0.07	495

* These blood samples were taken at 2 p.m.

† These blood samples were taken at 8:30 p.m.; most of the others at 8 to 9 a.m.

situations such as the stomach, bile pigment was only detected clearly 2 or 3 times in some hundreds of examinations. Blood or blood pigment was never in any case found in the urine, even when the intestine contained a great quantity of blood. The reaction of the urine whether passed during the period of health or while the animal was dying was always dis-

TABLE 2

Urine analysis

NUMBER OF DOG	COLLECTION DATE	TOTAL NITROGEN	UREA NITROGEN	URIC ACID	AMMONIA NITROGEN	CREATININE	
						Preformed	Total
		grams in 100 cc.	*grams in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*	*mgm. in 100 cc.*
1146	5–20	5.25	4.6	42	219	167	210
	5–23	3.04	2.1	38	149	161	333
	5–25	5.05	4.25	32	118	143	300
	5–26	2.64	2.0	43	94	328	500
	5–28	2.99	2.56	17	104	168	364
1182	5–20	3.5	3.1	17	120	91	150
	5–23	1.59	1.3	24	118	161	333
	5–24	2.02	1.51	39	111	200	273
	5–25	3.35	2.8	33	130	160	283
	5–26	2.22	1.7	35	131	215	370
	5–28	1.97	1.46	16	146	105	235
	5–31	1.36	1.03	trace	214	80	160
1195	6–8	5.6	5.2	36	176	204	300
	6–21	3.33	3.0	48	75	281	570
1197	6–5	1.89	1.45	40	59	151	333
1200	6–9	2.25	1.72	40	63	426	628
	6–21	3.5	2.5	53	130	308	571
	6–24	3.13	2.3	51	125	418	986
1203	5–17	3.1	2.7	38	273		
	6–5	2.3	1.43	39		333	400
1204	6–9	2.5	2.3	36	150	273	375
	6–21	1.27	1.07	21	40	133	230
	6–24	1.49	1.16	17	45	108	182

tinctly acid to litmus. The specific gravity of the urine varied within the usual limits. The color was normal. In short no indication was found that any disorganization of the kidney comparable to that of the gastro-intestinal mucosa had taken place. The question is of interest in connection with the increase of the non-protein nitrogen and urea in the blood,

for if excretion by the kidney was interfered with, this might be a retention phenomenon. Extracts from some of the protocols necessary for supplementing table 1 (and table 2) will now be given. The post-operative recovery after adrenalectomy was excellent in all the dogs.

Dog 1146. 11–16–25, right adrenal excised, body weight 8.9 kgm.; 3–31–26, 4–5, 4–17, 5–17, weights are 9.7 kgm., 9.8 kgm., 9.8 kgm. and 9.7 kgm. On 5–20 blood and urine examination. 5–21, left adrenal removed at 1:15 p.m. Body weight 9.7 kgm. On 5–23 and 5–25 (8:30 a.m.) blood and urine samples taken. Animal in excellent condition and eating well. 5–26 (a.m.) ate a good meal of bread and broth; blood and urine (2:00 p.m.); ate some meat; 6 p.m. in good health but seems to be getting less lively. Has not barked all day (as she usually does). On 5–27 head shakes from side to side, but she moves about actively when taken from cage; ate biscuit and meat in good amount, condition seems excellent except for shaking of head; at 3:30 to 4 p.m. had violent short convulsions, she walked just before without wobbling. At 4 p.m. blood and urine samples.

On 5–28 in the forenoon slight wobble in walking, 8:30 a.m. blood got, and later urine. Seems apathetic. At noon refused all food; lying on bed, though able to walk (wobble) when taken from cage. Much weaker than yesterday. At 3:30 p.m. is comatose; 4 to 4:30 p.m. several short convulsions, becoming more violent. Died at 4:30 p.m., following a short convulsion. Autopsy (at once). Thymus small; thyroid normal; liver congested; kidneys and spleen normal. Stomach contains bile; no congestion; small erosion near pylorus. Duodenum, moderate congestion becoming greater in lower half, with petechial hemorrhages. Lower part of jejunum strikingly congested and hemorrhagic; also large intestine, which contained feces with some blood. Uterus contained degenerated decidua.

Dog 1149. On 12–2–25, the right adrenal was excised. Body weight on this date 10.3 kgm.; on 3–3–26, 12.2 kgm.; on 4–5–26, 12.0 kgm.; on 5–17, 11.8 kgm. On 6–7–26 blood and urine examination. On 6–10–26, left adrenal excised at 2:00 p.m. Body weight 12.1 kgm. The dog continued in good health, and eating well. On 6–14, blood sample taken. On 6–15 (at 9 a.m.) appetite is keen, and condition good; at noon ate bread and broth and some meat and at 3:30 p.m. some rabbit. At 11:00 p.m. ate biscuit readily. On 6–16 at 8:00 a.m. ate biscuit as readily as usual, but at noon refused everything (biscuit, meat and rabbit). A stool was passed (12:10 p.m.) greenish (bile) and streaked with some blood. Is lethargic. On 6–17 blood taken. At noon refused food completely; stands a good part of the time and is strong on her feet; no wobble. At 4 p.m. vomited bile; lying on side; wagged tail but did not get up. At 7:30 p.m. found semi-solid brownish (tarry) feces with some blood. On 6–18 refused food; blood taken morning and evening; a little more tarry feces passed. She now wobbles when walking. At 5 p.m. very somnolent; eyes "mattery." Not comatose; same at 7:30 p.m.; lapping water but weak. At 8:45 p.m. liquid feces passed (on the bed) containing blood. When a blood specimen was taken the flow was slow; pulse 30 to 36 (rather irregular); this morning the rate was about 40 and more regular. There has been no coma up to this time. When put on her feet walks but with much wobble. At 10:15 p.m. deep coma; died about 10:30 p.m. Autopsy (at once). Stomach full of bile, no blood, no congestion; bile also in esophagus. Duodenum and upper part of jejunum contain blood and bile; ileum, colon and rectum down to anus congested and contain much blood. Pancreas much congested; spleen and kidneys not especially congested; liver congested, also omentum.

Dog 1182. 2–1–26, right adrenal removed; body weight 8.7 kgm. On 3–10, 3–26,

5–8 and 5–17 the weights were 8.0, 9.1, 10.3 and 10.2 kgm. On 3–18 gave birth to one pup; later nursed the pups of dog 1192 (paper III) when the mother died. On 4–12 some blood in stools. On 5–20 blood taken (8:30 a.m.). On 5–21 left adrenal removed (1:45 p.m.). Body weight 10.2 kgm. On 5–23 her condition was excellent; eating well; blood and urine examination. On 5–24 (9:00 a.m.) she had yelling fits and raced about in the cage. Blood and urine samples. At noon ate a good meal of meat, but continued to yell in the afternoon. On 5–25 (8:00 a.m.) she yelled when taken to room for blood and urine collection. At noon ate a good meal of bread and broth. At 8:30 p.m. she wobbles somewhat when walking but is active. On 5–26, she walks well; ate good meal of meat at noon, and later vomited, but otherwise seems very well. No more yelling. Blood and urine taken (2:00 p.m.). On 5–27 ate meal (meat) at noon. On 5–28 (8:30 a.m.) seems quite well; blood and urine examination. At noon ate good meal (meat) and retained it; very slight, if any, wobble when walking. On 5–29, blood examination (8:30 a.m.). In good condition all day. At noon ate good meal (meat). Walks without wobble. On 5–30, refuses food, but otherwise seems well. On 5–31 continues to refuse food; some wobble when walking; muscular twitching; blood and urine examination (11:30 a.m.); vomited bile, weaker. At 6:30 p.m., weak; very wobbly in walking. Died about 5 a.m. on 6–1. Autopsy. Liver normal in appearance; kidneys slight congestion; spleen mottled, normal size; pancreas much congested; stomach contained bile and blood, mucosa hemorrhagic; duodenum contents same as stomach, mucosa greatly congested and hemorrhagic; jejunum and ileum same as duodenum; large intestine moderately hemorrhagic mucosa, contents bloody.

Dog 1186. Young adult, probably virgin. 2–3–26, right adrenal excised. Body weight 8.5 kgm. Body weights on 2–19, 2–24, 3–2, 3–26, 4–5, 4–17, 5–8, 5–17, 5–26, 6–7, and 6–10 were 8.6, 8.6, 8.9, 9.4, 9.5, 9.8, 10.6, 10.4, 10.7, 10.4 and 11.0 kgm. On 6–7, blood sample. On 6–10, left adrenal excised (2:45 p.m.). On 6–11, very good condition; ate bread and milk readily. On 6–12, blood specimen (8:30 a.m.). Ate good meal of meat and biscuit at noon. On 6–13, ate dish of bread and milk (10:00 a.m.); ravenous appetite. Very active at 10:45 p.m., and ate greedily bread left by another dog. On 6–14, blood specimen; ate good meal of meat at noon. On 6–15, appetite keen; noon meal of bread and broth eaten very readily; keen for biscuit. On 6–16, blood specimen; ate noon meal (meat) very heartily. Up to this time her health has been excellent. On 6–17, blood specimen (8:30 a.m.); somewhat listless. Took her meal of bread and broth also biscuit, although she took longer time to eat it. In the evening took biscuit readily.

On 6–18, blood specimen (8:30 a.m.). Refused the noon meal (meat), absolutely, but took rabbit readily. Ran around in the hall; not wobbly on feet. Allowed another dog to enter her cage and eat up remains of her food (5 p.m.). At 10 p.m., she ate biscuit; searched perseveringly for a flea and drank a good deal of water. Her appearance is not bad.

On 6–19, blood specimen (9:00 a.m.). Head shakes a little; somewhat apathetic; ate a fair portion of rabbit readily and entirely, also biscuit. Refused noon meal (meat). She can walk (without wobble) but does not care to be much on her feet. On 6–20, blood sample (8:30 a.m.). She ate nothing all day. In morning came out of cage and wandered around with the other dogs; interested in other dogs; firm on her feet, but stands around more than the others. At 8 a.m. vomited a little (bile-tinged; a round worm in vomit). At 11:30 a.m. more bilious vomit, refused rabbit and all other food. At 9 p.m. semi-liquid stool on floor. Dog does not look happy; stretched out on bed; deep breathing; somewhat dazed when aroused; eyes mattery. At 11:00 p.m. unchanged.

On 6–21, at 6:30 a.m. found dead (cold). Probably died about 3 or 4 a.m.

Autopsy. Thymus large; liver, spleen and kidneys normal; pancreas markedly congested. Stomach contained some bile and worms, mucosa slightly congested and hemorrhagic. Duodenum and jejunum contain bile-stained liquid and many worms; ileum, mucosa congested and hemorrhagic, especially in the lower part in numerous small areas.

Dog 1188. Young adult. On 2–9–26, right adrenal excised. Body weights on 2–9, 3–10, 3–26, 4–5, 4–17, and 4–28, were 9.5, 9.8, 9.7, 9.5, 9.95, and 10.0 kgm. 4–27, blood sample taken (8:30 a.m.). On 4–28, left adrenal removed. On 4–29, ate bread and milk, condition very good. On 4–30, blood sample (8:30 a.m.); at noon ate its meal (meat) well; and the same on 5–1. Excellent health. At 9 p.m. on 5–1, it suddenly had a yelling insane fit; raced about the cage and later jumped over side of cage, raced about the room and tried to jump up on a window sill. After the two fits it seemed in very good condition, happy and wagging its tail. On 5–2, blood sample (8:30 a.m.). Seems perfectly well: no fits, ate noon meal of bread and milk. On 5–3 at 9:30 p.m. had a yelling fit; otherwise seems all right; blood specimen obtained; dog refused meat at noon. On 5–4, yelling frequently all day. Blood sample taken (9 a.m.). At noon ate a few small bits of bread soaked in broth but left most of the meal. Getting somewhat wobbly on hind legs, but stands around and tries to get out of the cage, as if alarmed. At 5–5, blood taken (9 a.m.). Dog lies on side jerking, not unconscious but dazed. At 10 a.m. in coma; at 11 a.m. died. Autopsy at once. Liver congested; pancreas markedly congested; spleen normal; kidneys congested. Stomach filled with bile, no congestion, 3 or 4 small ulcers at pyloric end. Duodenum contained blood, bile and worms, marked congestion of mucosa, two very large deep ulcers at pyloric end. Jejunum and ileum contained many round worms, also bile and blood throughout, the mucosa was congested and hemorrhagic throughout. Rectum, mucosa much congested and hemorrhagic; rest of large bowel not congested. It may be noted that the dog did not at any time vomit, or pass bloody stools.

Dog 1189. Young adult male. On 2–9–26, the right adrenal was excised. Body weights on 2–9, 3–10, 3–26, 3–31 and 4–5 were 8.0, 8.3, 8.4, 8.2 and 8.3 kgm. On 3–29 a blood sample was taken. On 3–31, the left adrenal was excised (2 p.m.). On the next day bread and milk was taken and thereafter the usual noon meal. He behaved in all respects like a normal dog up to and including 4–4, in excellent health except for mange. On 4–3, a blood sample was taken. On 4–5, he had a yelling and racing fit twice in the forenoon and another in the afternoon. He took his meal at noon quite well, and seemed in excellent condition except for the yelling fits. On 4–6, in the morning, bile-stained vomit was found in the cage. He had not vomited when visited last night. Refused noon meal completely, loose bowel movement (dark). Vomited bile twice this afternoon. Blood sample taken in the evening (8:30 p.m.).

On 4–7, took no food; vomited bile and passed some bloody stools. No asthenia. Blood sample taken. On 4–8, in the morning he is getting weak, very apathetic; vomited bile. In the afternoon somnolent; wobbly on his feet. Died at 7 p.m. Autopsy at once. Stomach, mucosa very hemorrhagic, contents bile and blood. Duodenum and nearly the entire small intestine congested and hemorrhagic; many worms; bloody contents; ileum less than upper part. Large intestine quite hemorrhagic, with bloody contents. Pancreas intensely congested. Liver and kidneys moderately and spleen slightly congested.

Dog 1195. Fox terrier. On 2–17–26 right adrenal excised. Dog is mangy. Body weights on 2–17, 2–19, 2–24, 3–2, 3–10, 3–20, 3–26, 4–5, 6–7 and 6–19 were 7.5, 6.4, 6.7, 7.0, 6.7, 6.9, 6.8, 8.5 and 8.9 kgm. On 6–8, blood and urine samples collected. On 6–19, left adrenal removed (10:40 a.m.). On 6–20, at 6:30 a.m. some vomit

was found on floor, containing a round worm. He had no food since operation. At noon ate some rabbit, biscuit and bread with milk. On 6-21, blood and urine obtained at 8:30 a.m. Dog lies on bed, not weak, but seems out of sorts. Took no food today. Vomited small amount of frothy white material. At 8 p.m. more vomit with bile. Apathetic. 6-22. Blood and urine obtained (8:30 a.m.). At 10:30 a.m. clear acid liquid vomited (no bile). Took no food today. At noon vomited bile. At 7:30 p.m. vomited clear acid liquid with mucus flakes. Semi-solid feces containing some blood. 11 p.m., unchanged. Strongly acid liquid vomited (no bile); must have come off bed to vomit. Dog lethargic; does not look well.

6-23. Found dead at 6 a.m. (cold). Autopsy. Entire right lung very much congested, no consolidation; left lung moderately congested. No apparent congestion in liver, little in spleen. Some blood stained exudate at site of left adrenal. Infection in wound but not extending beyond peritoneum. Left kidney congested, right normal. Stomach contained much bile, mucosa moderately hemorrhagic, 4 or 5 small ulcers at pylorus. Duodenum same as stomach, a few small ulcers and a larger one at pyloric end. Jejunum and ileum, moderate congestion of mucosa, less in ileum than in jejunum, contents bile, blood and worms, a large hemorrhagic patch about 4 inches long at lower end of jejunum. Pancreas intensely congested.

Dog 1196 (partly collie). 4-15-26, right adrenal excised. Body weight 11.3 kgm. 4-16, put in quarantine, coughing badly. 4-26, coughs less, taken from quarantine. 4-29, blood specimen taken. 6-8, blood and urine specimens obtained. 6-11, in morning vomited a considerable amount of bile but at noon ate a large meal of meat. General health good. 6-23, left adrenal removed (1:30 p.m.). Body weight 12.7 kgm. 6-24, 6 a.m. in excellent condition; took biscuit eagerly. Appears quite the same as before operation. Ate noon meal (bread and broth). At 8 p.m. has vomited all her food; liquid, strongly acid to litmus. 6-25, blood samples taken (8:30 a.m.). At 11 a.m., not lively, lay on bed, refused noon meal (meat). Ate ½ biscuit, but not readily.

6-26, blood taken (8:30 a.m.). At 11 a.m. refused meat and biscuit. Lying on bed, much less energetic than before the second operation. At 1:45 p.m. refused ham and biscuit completely. Would not come out of cage. Some liquid stools with blood (tarry) and bile. Came off bed to micturate and did so copiously. At 2 p.m. vomited bile-stained mucus. At 5:30 p.m. more green vomit and liquid stool containing blood (tarry), recognized by spectroscope. At 7:30 p.m. she came out of cage. At 8:30 p.m. took blood sample; very slow flow. Vomited (bilious matter, strongly acid) while the blood was being collected. At 9:30 p.m. more feces with blood. 6-27, 2 a.m., loud yelling from time to time (every 5 or 10 minutes) with convulsive progression movements of fore and hind limbs. The movements, but not the yelling are easily induced by striking head or other part of body. Dog unconscious or sometimes semi-conscious. More feces similar to those passed before. No yelling after 3 a.m. Died at 6:45 a.m. Autopsy at once. Liver and kidneys marked congestion, spleen moderate. Stomach, mucosa moderately congested and a number of small ulcers at pylorus. Contents, bile, worms and blood (many small blood clots). Duodenum same as stomach, but more hemorrhagic congestion; a number of hemorrhagic areas and an ulcer near the orifice of the pancreatic duct. Pancreas greatly congested. The lower portion of jejunum and entire ileum contained large amounts of blood, increasing from above downward; mucosa congested and hemorrhagic. Cecum and its process contained bile and some feces but no blood, and there was no congestion. Colon and rectum, congestion especially on the longitudinal ridges; some blood and bile in contents. Subcutaneous stitch abscess in wound extending into muscle, but not to peritoneum.

Dog 1197. Poodle. Was pregnant when right adrenal was removed on 4-15-26. Body weights on 4-15, 4-17, 5-8, 5-17, 5-26 and 6-2 were 8.9, 8.8, 7.8, 7.8, 8.0 and 7.9 kgm. On 4-18, 3 pups were born. On 4-29, the dog being in good health and eating well, a blood sample was got. On 6-2, left adrenal excised (2:30 p.m.). On 6-3 and 6-4 ate well. On 6-5, blood and urine taken in morning. Slightly apathetic. Refused food at noon. At 5:30 p.m., lying on side, seems weak. On 6-6, died about 7 a.m. Autopsy. Pancreas marked congestion, omentum congested. Stomach contained about 50 cc. of liquid (water and mucus); duodenum bile-stained mucus, mucosa slightly congested in both. Jejunum, no congestion, many worms, bile-stained mucus. Ileum, moderately congested and hemorrhagic at lower end with some hemorrhagic patches higher up. Liver, kidneys and spleen normal. Some extravasation of blood below left kidney.

Dog 1200. Wire hair mongrel (terrier). 4-23-26, blood for analysis. 6-2, right adrenal excised. Body weights on 5-8, 5-17, 5-26, 6-7 and 6-19 were 9.7, 10.0, 9.7, 9.7, 9.3 and 9.35 kgm. On 6-9, blood and urine samples taken. 6-19, left adrenal excised (10:15 a.m.). Dog was in excellent health, eating well. 6-20, in morning took large amount of water and vomited; later ate biscuit, rabbit and bread and milk. Good condition. On 6-21, blood and urine taken about 8:30 a.m. Ate noon meal. On 6-22, unchanged, eating well, active.

6-23, not as active, refused noon meal (meat); blood sample taken at 8:30 a.m. and another at 8:30 p.m. At 6 p.m. came out of cage, and took a very small piece of rabbit. Walked well. At 11 p.m. solid stool (blood stained). Has not touched food (rabbit) left in cage.

6-24, blood and urine got (8:30 a.m.). Ate nothing today, but looks better and more alert; came out of cage and walked well. Seems strong enough, but no desire for exertion. 6-25, unchanged. Refused all food. Blood sample taken 8:30 a.m. At 11 p.m. semi-liquid feces on floor with blood (tarry).

6-26, blood specimen (8:30 a.m.). Small stools with blood (tarry) were passed at different times. Walked well (10 a.m.), but no wish to do so. At 10:30 a.m. seems weak. At 1:45 p.m. comatose; has passed feces (tarry) containing blood and bile. Tongue cyanotic. At 1:55 p.m., a few gasps, and death. Autopsy at once. Liver much, kidneys moderately congested; spleen normal; pancreas decidedly congested. Stomach distended with bile-colored fluid (some small blood clots on mucosa, which is not greatly congested). Duodenum, more blood in contents and mucosa moderately edematous and hemorrhagic. Jejunum and ileum full of blood, mucosa congested and hemorrhagic. Large intestine including cecum and its process the same.

Dog 1204, 6-2-26, right adrenal excised. Body weight 12.5 kgm. Blood and urine samples taken 6-9, when the dog was in excellent condition. On 6-19 left adrenal was removed (11:15 a.m.). Body weight 12.7 kgm. 6-20, eating well (bread and milk), active. Same on 6-21, when he took biscuit readily in morning and all the noon meal (meat). Blood and urine samples taken in morning.

On 6-22, less lively; took morning biscuit and half the noon meal (bread and broth). Refused meat but ate 2 biscuits and a good portion of rabbit, chewing the bones thoroughly. At 4 p.m. refuses to come off bed; labored, jerking respiration; head wobbles slightly. At 11 p.m. unchanged except that twitching is increased (head and limbs); the movements are synchronous with the slow deep respiration. Drowsy, not comatose.

6-23, at 6:30 a.m. seemed better, but refused biscuit. Refused meat at noon but ate a good portion of rabbit readily. Blood samples at 8:30 a.m. and 8:30 p.m. At 6 p.m. came out of cage; wobbly; stands with legs apart. At 9 p.m. vomited large amount of partly digested food (brown, semi-liquid).

6-24, took no food today, otherwise the same as yesterday, walks fairly well, licked himself several times. Blood and urine samples taken in morning.

6-25, 6 a.m. unchanged, walked into another room. No food taken today. At 11 a.m. in very poor condition; can stand with legs apart, but holds the head down. No interest in anything. At 4 p.m. lying on belly with hind legs spread out quite unable to rise. Eyes full of matter. Conscious. Died about 10 p.m. in same position. Autopsy at once. Pancreas, liver and kidneys much congested; spleen not. Stomach full of bile, one worm; moderate congestion with one or two hemorrhagic spots. Duodenum congested and some blood on mucosa. Jejunum more congested and hemorrhagic than duodenum; worms and bile everywhere. Ileum congested and hemorrhagic, especially in upper portion; blood and bile in lumen. Ileo-colic valve quite red. Moderate congestion of colon and rectum. Omentum congested.

It may be mentioned that in a typical case of Addison's disease which came under our observation at St. Alexis hospital two blood analyses were made, one when the patient was in poor condition, the other when he was better. The non-protein nitrogen was somewhat, the urea nitrogen much, less when the patient was in better condition. He was about 40 years old. Pigmentation etc. had existed over 2 years with asthenia coming on at intervals. He has tubercular hip disease. On 5-18, asthenia was considerable, pigmentation very well marked. The systolic pressure was 66, the diastolic 48. In the blood the non-protein N was 52.5; the urea N 33.4; uric acid 4.4; preformed creatinin 1.0; total creatinin 3.1; amino-acid N 9.2; and Cl expressed as NaCl 410 mgm. per 100 cc. The blood sugar was 0.11 per cent. In the urine (the first specimen voided in the morning) the total N was 1.75 per cent; urea N. 1.66 per cent; uric acid 49 mgm.; NH_3 106 mgm.; preformed creatinin 118 mgm.; total creatinin 312 mgm. per 100 cc.

On 6-23, he was ready to leave the hospital. The blood pressure was 93 systolic, 76 diastolic. In the blood the non-protein N was 46; urea N 15; uric acid 4.4; preformed creatinin 1.2; total creatinin 3.8; amino-acid N 8.0; and Cl expressed as NaCl 430 mgm. per 100 cc. The dextrose was 0.091 per cent.

Serum percentage, etc. More physical factors, as the conductivity of the serum, relative volume of the corpuscles and plasma (and therefore viscosity), specific gravity and refractive index of serum (serum proteins), erythrocyte concentration, Hb concentration were studied on 20 adrenalectomised dogs. The results have been summarised in a table published elsewhere (Stewart, 1926). They must now be considered more in detail and in their proper setting in connection with the chemical changes just described. Some illustrative protocols must also be given to enable the reader to fix the time of onset of the physical changes with respect to the clinical condition of the animals, and to the chemical changes in the blood. Three of the dogs in the group of 20 mentioned were pregnant, viz., dogs, 996, 1034, and 1036 and will be alluded to in paper III.

The most characteristic change observed is an increase in the volume of erythrocytes relatively to serum. This is common, perhaps constant, towards the end. Often it may not be observed till the last day or the day before death. Sometimes the concentration of the blood seems to begin to increase even in the period of good health towards its termina-

tion, as if presaging the fatal change. But the greatest concentrations are not reached till near the end. Then they are often extreme, and the wonder is how the blood can still run. As a matter of fact at this stage the blood flow is often very slow. This is due, of course, largely to inefficiency of the heart, but the great increase in the viscosity of the blood occasioned by the augmentation of the relative volume of the erythrocytes is not a negligible factor, particularly as it may lead to stagnation of blood in areas like the splanchnic region, where the small vessels may be blocked by plugs of erythrocytes slowly moving or not moving at all. This may be a factor in bringing about the hemorrhagic congestion in the mucosa of the gastro-intestinal tube. The increased concentration of erythrocytes is also, of course, revealed by an increased erythrocyte count, an increased Hb percentage, an increased specific gravity of the blood, and a diminution in its electrical conductivity. While the serum is decreasing in amount, and sometimes enormously, it does not seen as if the total solids of the serum (i.e., especially the proteins) increase in proportion to the total solids of the blood. But more work is needed on this point. The electrical conductivity of the serum, indeed, shows, if anything, a tendency to diminish as the concentration of the blood increases, but this seems to be sufficiently accounted for by the small diminution of chlorides already discussed, with which it runs a fairly parallel course and agrees fairly well in magnitude.

Discussion. Whether the diminution in the chlorine is related to a continued and perhaps increased secretion of HCl in the stomach of an animal which has stopped eating, this secretion being perhaps part of the mechanism by which the poisons, if there are poisons, are being combated, it would be premature to discuss. As the blood loses much water during the stage of concentration, it must lose salts also, and Cl, under certain conditions may pass out in a somewhat greater proportion than the water. The loss of water may be thought of as related to an irrigation of the tissues and of certain eliminating organs, especially the gastro-intestinal mucosa, possibly the kidneys, so as to get rid of the poison. The observations on the life-prolonging effect of intravenous injections of salt solutions, which will be dealt with in paper IV lend considerable support to the idea that some such process may be important. The injections would in any case dilute the blood and so diminish its concentration and also dilute the poisons. It is at or about the time when the non-protein nitrogen, the urea nitrogen and the undetermined fraction of the non-protein nitrogen tend to reach their highest values that the blood concentration tends to reach its maximum. Also just as in many cases the non-protein nitrogen, etc., may begin to increase before the serious symptoms have declared themselves, so there may be a prodromal increase in the blood concentrations at or about the same time as that in the nitrogen. The increase in the nitrogen cannot

be due merely to the concentration (loss of water) of the blood. For first the nitrogen increase is far too great, and secondly other constituents (uric acid, Cl) are not increased. Whether the increase of non-protein nitrogen is due to retention or not, whether it is in itself an essential part of the intoxication, which is perhaps unlikely, it seems reasonable to assume, from the time of its occurrence, that it is associated in some way with the breakdown of the mechanism which has hitherto enabled the adrenalectomised dog to carry on. The concentration of the blood may be considered as another concomitant change. Whether, as suggested, it is due to the call of the eliminating organs whatever they may be, for water to help to carry off the poison, it is not difficult to see that, once started, the process may be self-accelerating. For if increase in the proportion of erythrocytes facilitates stagnation, especially in the splanchnic area, it is commonly assumed that such stagnation tends to cause a loss of water from the blood.

In dog 1018, just before excision of the second adrenal, the blood count was 6,880,000; blood sugar, the previous day, 0.103 per cent. Two days after the operation, the dog being in good health and eating well, the count was 6,550,000, and the sugar 0.105 per cent. The conductivity of the serum was $K\ (25°) \times 10^4 = 124.3$; the serum constituting 48.9 per cent of the blood (conductivity method, hematocrit 48.5 per cent). Four days after the operation, the dog still being very well, and the appetite good there was no change in the concentration (serum 48 per cent by the hematocrit). The blood sugar was 0.08 per cent. Seven days after the operation, food having been definitely refused the previous evening, the serum constituted only 41.2 per cent of the blood; the specific gravity of the blood was 1,067. The dog was apathetic, vomited occasionally, and wobbled in walking. Seven hours later she was dead. The blood concentration seen in this animal was moderate but definite and probably not evident till the last day of life. Some water was being taken, and the small quantity of liquid vomited could not account for the loss of water by the blood. It is perhaps worth remarking that the adrenalectomised dogs generally continued to drink water until very near the end. Although we have no quantitative data it seemed as if they took at least as much water as normal animals.

In dog 1025 we have an instance of an animal which showed a decided increase in blood concentration in advance of the advent of symptoms. The results are most easily compared with those in the cases where non-protein nitrogen, etc., were estimated, when given in the form of a protocol.

Dog 1025, fox terrier. On 4–30–25 right adrenal excised. Body weight 7.3 kgm. 6–4, erythrocyte count 7,740,000, Hb 100 per cent, blood sugar 0.10 per cent, conductivity of serum 132.0; percentage of serum in blood 58.8; calcium 13.0 mgm. per 100 cc. serum. 6–5, excised left adrenal (2:45 p.m.). Body weight 9.05 kgm. 6–8, active and eating well; blood sugar 0.108 per cent; conductivity of serum 127.2;

percentage of serum 49.6; calcium 11.6. 6–10, dog in good health, and eating well. Erythrocytes 9,100,000; Hb 120; blood sugar 0.105 per cent; calcium 14.3 mgm. per 100 cc. serum; conductivity of serum 120.0; percentage of serum 38.6; 6–12, eats well and is active, very playful as usual; blood sugar 0.118 per cent; Hb 122 per cent; conductivity of serum 120.0; percentage of serum 38.1; calcium 15.2 mgm. 6–13 refused all food today; stretching and yawning; playful but not so obstreperous. 6–14, taking no food; less active; eyes dull. At 9 a.m. blood taken; blood sugar 0.083 per cent; Hb 128; conductivity of serum 117.8; percentage of serum 35.2; specific gravity of serum 1,026; calcium 17 mgm. in 100 cc. serum. At 5:45 p.m. found vomit (with bile) for the first time. Weak. At 11 p.m., very weak, unable to stand. Vomited a large amount of liquid (with bile) strongly acid to litmus. Severe retching, feces with blood. For the next hour, moderate convulsive (progression) movements with an occasional whine, semi-comatose; pads of feet cold. 6–15, 12:10 a.m. passed several small stools with blood. Walked 8 or 10 yards, though tottering; did not avoid obstacles. At 1 a.m. comatose. Progression movements occasionally, also opisthotonos. At 3 a.m. died. *Autopsy at once.* Stomach congested and full of dark brown liquid with blood pigment (hematin). Pyloric end of stomach was invaginated into cardiac end, perhaps during retching; this was not seen in any other of our dogs. Small intestine much congested and hemorrhagic in its whole length, and with bloody contents and clots on the mucosa. Large bowel to anus congested, hemorrhagic and with bloody contents. Cecum has blood in the contents. Pancreas much congested; mucosa around orifice of common duct red. Liver fairly congested; spleen not congested.

In this animal there was a rather abrupt increase in the concentration of the blood about 3 days before refusal of food, becoming still greater on the day before death. The same thing is seen in dog 1037, and is still more marked in 1038. The final concentration is the greatest observed in any of the animals.

Dog 1037. On 6-2-25 the erythrocyte count was 7,800,000; Hb 108 to 110; blood sugar 0.084 per cent; serum conductivity 128.8; serum percentage 47.6. On 6-3-25 left adrenal excised. Body weight 8.35 kgm. 6–11, blood sugar 0.091 per cent; calcium 12 mgm. in 100 cc. serum; conductivity of serum 128.2; percentage of serum 48.3. 6–12, right adrenal excised in 8 minutes (2:30 p.m.). On 6–13 in good condition; eating well, came out of cage. Same on 6–14 and 6–15. On 6–15, the blood sugar was 0.085 per cent; erythrocyte count 8,000,000; Hb 118; serum calcium 14 mgm. in 100 cc.; conductivity of serum 124.9; percentage of serum 41.3; specific gravity of blood 1,0644, of serum 1,0239. 6–16 to 6–18, health and appetite good. On 6–19 he was as active as ever in the morning but refused food. In the evening less active, lying more on his bed, although able to walk well. At 2 p.m. the erythrocyte count was 9,700,000; leucocyte count was 11,700 (about the same as on the other days); Hb 126; percentage of serum 29; blood sugar 0.074 per cent. On 6–20 took no food. At 11 a.m. is quiet and "stretches" a good deal; refused food. At 9:30 p.m., bloody stools and bloody liquid on floor. Vomit (with bile). On 6–21 quite asthenic; diarrhoea (some blood). 6–22, died during night. Autopsy. Stomach congested considerably and contained bile, worms and blood pigment; duodenum same as stomach except that congestion was less; jejunum quite congested and hemorrhagic; contents more bloody. Ileum and colon slightly congested in patches; rectum more congested and hemorrhagic.

In dog 1037 the serum percentage began to diminish somewhat on the third day after the second operation. On the 7th day after the operation the serum reached the low volume of 29 per cent. This indicates very concentrated blood for a dog, as does the low conductivity of the blood (K (25°) $\times 10^4 = 23.2$). Symptoms had then definitely appeared, and death occurred $2\frac{1}{2}$ days later. For various reasons observations could not be made between 6–15 and 6–19, an interval during which it must be assumed that the concentration went on increasing. In dog 1038 the observations were better spaced. The diminution in the percentage of serum was already marked 3 days after the second operation and 6 days before death. At this time and for 3 days longer the dog was in excellent health and was eating normally. Yet the concentration of the blood was steadily increasing, the minimum value of the serum percentage (23.7) being attained 3 hours before death. The blood conductivity showed the extraordinarily low value of K (25°) $\times$ 10^4 = 17.1.

Dog 1038. 6–2–25; blood sugar 0.09 per cent; leucocytes 18,000; erythrocytes 7,200,000; Hb 108; conductivity of serum 132.4; serum in 100 cc., blood 51.1 cc. 6–3, left adrenal excised. Body weight 9.15 kgm.

6–10 (morning), blood sugar 0.09 per cent; calcium 11.4 mgm. per 100 cc. serum; erythrocytes 6,400,000; leucocytes 10,200; Hb 106; conductivity of serum 132.5; percentage of serum 50.6. Right adrenal removed (1:30 p.m.) in 10 minutes. Body weight 8.55 kgm. 6–12, eating well. On 6–13 in good condition and eating well. Blood sugar 0.091 per cent; calcium in serum 13 mgm. in 100 cc.; erythrocytes 10,200,000; leucocytes 14,250; Hb 130; conductivity of serum 123.7; serum percentage 37.9. On 6–14 ate well. 6–15, ate her food. At 7 p.m. in good condition but stretches a good deal.

6–16, good condition. Ate biscuit and meat. Wound healed by first intention. Blood sugar 0.085 per cent; Ca 12.5 mgm. in 100 cc. serum; conductivity of serum 116.0; percentage of serum 34.4; specific gravity of blood 1,0702, of serum 1,0252.

6–17. Took some food (biscuits and meat) but only by coaxing and hand feeding. Somnolent. At 9 p.m. lapped much water; in a minute or two vomited at least 150 cc. of watery liquid with a few whitish flakes, decidedly acid to litmus. She lapped up some of the vomit again.

6–18. Took no food today. Desires petting. At 8 a.m. passed stool containing blood. At 11 a.m. in poor condition but stands up when called, and even walks around. Blood sugar 0.058 per cent; Ca 17 mgm. in 100 cc. serum; Hb 128; serum conductivity 116.6; percentage of serum 29.1; specific gravity of blood 1,071, of serum 1,0233. At 5 p.m. lying on bed; has passed some liquid feces with blood. Walks well and without wobble when taken out of cage, not weak, though hind legs more widely separated than normal. At 10 p.m. more vomit.

6–19, 8 a.m., came out of cage but quite wobbly; took no food; asthenic. At 2 p.m. blood sugar 0.07 per cent; Hb 122; serum 17.1 cc. in 100 cc. blood; conductivity of blood K (25°) $\times$ 10^4 = 17.1. Died at 5 p.m. quietly. Autopsy at once. Liver, spleen and kidneys congested. Stomach, mucosa congested and hemorrhagic; 1 large and 3 small ulcers at pyloric end; contains 25 cc. almost black liquid containing blood and bile. Duodenum moderately congested, and contained reddish brown liquid (bloody). Jejunum and ileum much congested, with bloody contents throughout. Large intestine not so much congested. Pancreas greatly congested.

Lest the reader should get the impression that concentration of the blood is invariably found in dogs dying after adrenalectomy, we shall finish the description of this portion of the experimental material by giving an instance (dog 1020) where no concentration was found, although observations were continued within 36 hours of death when serious symptoms were already present. Certainly these cases are less frequent than the type previously described, but they exist. It is probable that samples taken nearer the end would show a concentration in these cases also. But the absence of concentration when characteristic symptoms are already well developed indicates that the concentration of the blood may be only a concomitant and not a causative factor in the production of the symptoms. It may or may not have a bearing on the exceptional result in dog 1020 that the percentage volume of serum in the initial observation was the highest seen in the male or non-pregnant female animals examined after adrenalectomy (about 64 per cent). The highest percentage seen in any of our animals was in a pregnant bitch during the period of good health (77.5 per cent). If an animal is habitually running on a high serum percentage before adrenalectomy it may be more difficult for this percentage to be depressed.

Dog 1020, bull terrier. 4-27-25, right adrenal excised. Body weight 6.85 kgm. 5-13, blood sugar 0.10 per cent; 5-14, erythrocytes 6,320,000; leucocytes 12,800 (morning). At 2:15 p.m. excised left adrenal. A good deal of hemorrhage from an artery, probably renal, and trauma, but excellent post-operative recovery. Body weight 6.7 kgm. 5-16, very good health, eating well. Blood sugar 0.095 per cent; erythrocytes 6,240,000; leucocytes 12,480; conductivity of serum 126.2; percentage of serum 63.9. 5-18, eating well; very active. Blood sugar 0.077 per cent; erythrocytes 6,440,000; Hb 90; leucocytes 13,000; serum conductivity 120.0; percentage of serum 62.4. 5-18 and 5-19, condition very good, eating well. Blood sugar on 5-20, 0.085 per cent. 5-21, body weight 6.75 kgm. Eating well, active; (10 p.m.), barked loudly at her reflection in a window.

5-22. Less active; yawning, stretching and lying about; took some meat only with coaxing; later refused all food.

5-23, refused food; stretching and yawning; walks a little (no wobble). At 1:30 p.m. salivating; after drinking water vomited bile. Blood sugar 0.074 per cent; erythrocytes 6,250,000; Hb 90; leucocytes 17,000; serum conductivity 119.5; percentage of serum 61; specific gravity of blood 1,058. At 3:30 p.m. walks with wobble, getting weaker.

5-24, refused all food. 8 a.m., has vomited a good deal of (bilious) liquid, weakly acid to litmus. No change throughout the day. Does not appear to have any pain or nausea. Shifted to different parts of the room. Not specially weak although some wobble. At 7 p.m. more vomit, same as before. Died about 11:30 p.m. A little blood stained liquid on bed and on anus. Autopsy immediately. Much congestion of gastro-intestinal mucosa from cardiac end of stomach to anus, specially upper two-thirds of small intestine; bloody contents everywhere, especially in small intestine. Stomach contents on standing separated as is common, into a top (greenish) layer, giving a strong Gmelin test, a middle (the largest) layer, reddish

and containing much blood pigment. The bottom layer contains the suspended matter. Pancreas much congested. Liver, spleen and kidneys not specially congested.

SUMMARY

In the adrenalectomised dogs (male and non-pregnant females) the non-protein nitrogen of the blood showed a marked rise when the characteristic symptoms, especially refusal of food, developed. The increase became greater as time went on up to death. An increase in the urea nitrogen is the great factor in augmenting the non-protein nitrogen, but the "undetermined fraction" also increases, often very greatly. There is no significant change in the uric acid, sometimes a relatively small increase in the creatinine and the amino-acid nitrogen. The blood sugar percentage is not materially altered until near the end, when a moderate diminution may be found. In a few instances in which the serum calcium was determined it seemed to be somewhat increased with the onset of the symptoms. The blood Cl is often moderately diminished.

The increase in non-protein and urea nitrogen is often found to precede the appearance of serious symptoms, coming on when the animal seems to be in good health and heralding the breakdown which is soon to come. The undetermined fraction may also show some increase at this time.

A concentration of the blood, with increase in solids, specific gravity, Hb percentage, erythrocyte count and relative volume of erythrocytes can be detected in most cases somewhat in advance of the onset of the symptoms. The change then goes on increasing till death. Roughly speaking, this change is coincident with that in the non-protein nitrogen. Possible relationships of these two sets of changes to each other and to the development of the clinical picture are discussed.

Similar changes both in non-protein nitrogen and in blood concentration were seen in pregnant dogs. But since pregnancy is associated with a great prolongation of the survival period after adrenalectomy, an effect which requires separate discussion, these observations are described in a separate paper (paper III).

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Contents of Volume VI, 1926

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